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RECORDS of RESEARCHES

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UNIVERSITY of TOKYO

No. IX (1958-1959)

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Contents

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I. Agriculture

Abstract Nos. 1—23

1. **Studies on the sterility in rice plant induced by superabundant nitrogen supply and insufficient light intensity.** Yosiji TOGARI, Yasumitsu KASHIWAKURA (Proc. Crop Sci. Soc. Jap. 27, 3-5, 1958)

Paddy rice plants, variety Nōrin No. 29, cultured under various light intensities and levels of nitrogen were examined for sterility. The results obtained are summarized as follows:

1) The occurrence of sterility increased, associated with an increase in the level of nitrogen and a decrease in the light intensity during a month prior to heading.

2) A negative correlation was consistently observed between the occurrence of sterility and number of pollens on a stigma counted about 1.5 hours after flowering.

3) It was observed that the sterility under high levels of nitrogen and low light intensities was caused by inhibition of pollination resulted from incomplete dehiscing of anthers and abnormal behaviour of filament at the time of flowering.

2. **Studies on some indications of potassium deficiency in rice plants.** Yakichi NOGUCHI and Tomota SUGAWARA (Potassium Symposium [Japan] 1957. 14-26, 1958)

There found to be so many hunger signs of potassium in rice plants. Among them, the following three, i.e. brown spots on leaves, lack of starch accumulation in the cells of stems, and decrease of the amount of starch contained in pollen have mainly been studied.

In the condition of potash deficiency, little brown spots first represented on the lower leaves of rice plants, and then they extended to upper ones by and by. Of course, the degree of symptom denoted by both the number and shape of spots varied according to varieties and conditions of culture. No ring-formed figure was found in the spot, and the occurrence of necrosis was proved by the microscopic observations. The cells of spot were only filled with brownish substance and showed neither shrinking nor disintegration. The spots were apt to appear in the row of stoma cells near vascular bundles and in vascular bundles, and they were especially rare in motor cells.

There was, however, several cases, in which no spot was seen even though the plant was cultured under the condition of potash deficiency. Defect of nitrogen or iron in soil or solution produced few brown spot. Same fact was found, when paddy rice grew in insufficient supply of water. Chemical analysis proved, in general, the level of potash content in the leaves was low when rice plants were hunger in potassium. This tendency was intensified by increase of manganese and iron content in leaves. From the results, it is considered that the appearance of brown spots related to the iron content in leaves; so that it may be very interesting, that spot is non-apparent under potash deficiency, if the spot presentes with the production of brown substance induced by oxdation of polyphenol compounds and the oxdase concerned is cytochrome, in the authors' expectation.

The content of Amid-type nitrogen and especially that of NH_4-type nitrogen were

generally very high in the leaves of potash-starved plant, but total nitrogen was not influenced by the amount of potash supply, therefore it may be supposed that the extraordinary increase of NH_4-type nitrogen was one of the causes, which produce the sudden weathering of plant, an indication of potash deficiency.

The starch contained in pollen acts an important role in the fertilization for germination of pollen and elongation of pollen-tube. In spite of having such important action, starch is very scanty in pollens of potash deficient plants. Activities of phosphorylase and β-Amylase, which have close relation to desolution of starch, was examined, and the fact was found that the activity of the former was very weak, and that of latter, on the contrary, very strong. So, the lack of starch accumulation may be originated by combination of decrease amount of carbohydrate, which is row material for formation of starch, strong activity of β-Amylase and interference of the action of phosphorylase.

3. **On the mutations induced by water culture under the condition of phosphorus shortage in rice plants.** Yakichi NOGUCHI (Jap. Journ. Breed. 8, 137–141, 1958)

A few investigators have hitherto reported that they succeeded to increase mutability of plants by keeping them under an abnormal condition of nutrition excluding a special chemical element, such as nitrogen, phosphorus or sulphur. Recently, the chemical analysis proved phosphoric acid was one of the essential components of nucleic acid, which was believed to be an heritable substance. Therefore, the effect of phosphorus shortage on the composition of nucleic acid, and consequently induction of mutation was examined.

Progeny test of the lines of rice plants originated from the seeds of plants which were cultured in nutrient solution wanting in phosphorus for whole the life except a few weeks at the seedling stage have progressed in these several years and a good number of variations, such as chlorophyl defect, poor growing habit, gigas-type, sterility etc., was found, but most of them were mere modifications and disappeared in the later generations.

Five mutants—variegation, awned, dwarf-type, sterility and incomplete emergence of ear—were, however, obtained after breeding for the hereditable characters in question. Incomplete emergence of ear is a new character in rice plants and it calls our attention for its practical value. Grains ripe on the erect ear the whole stem remaining in the leaf-sheaths and the ear scarcely move even in the strong wind, so the character may contribute to escape from the injury of typhoon which visits very often at the flowering time of rice plants.

4. **Studies on the control of flower bud formation by temperature and daylength in rice plants. Part I. Hastening of heading by high temperature.** Yakichi NOGUCHI (Jap. Journ. Breed. 8, 248–254, 1959)

Both temperature and the better condition of nutrition, especially the former, help the plants grow rapidly in six early-maturing varieties of Japanese rice. The high temperature had, moreover, a favourable effect on the formation of flower buds, consequently the emergence of ear was remarkably hastened accompanied with developing fewer leaves per plant. The influence of nutritive elements on the number of leaves per plant was tested using "Norin No. 11", an extra-early-maturing variety and it was proved that the amount of nitrogen supplied controlled the speed of developing

leaves unrelated to the heading. The more nitrogen was given, the larger the number of leaves was per plant. The stage of development, at which the plants were most sensitive to high temperature for induction of their flower buds, was clarified by the response to form flower buds and was determined that the stimulation did not occur at the early stage of germination before development of the third leaf.

Part II. Determination of the stage affected by high temperature for induction of flower bud. Yakichi NOGUCHI and Etsuo KAMATA (Ibid. 9, 33-40, 1959).

It was found in the test of Japanese rice that early sowing generally resulted in early heading, but the early-maturing varieties were apt to accelerate their heading when they were cultured under high temperature (above 25°C.). Under natural conditions the temperature rose day by day during early summer and it gave a good effect upon the formation of flower bud in the early-maturing varieties. So it can be said that high temperature is one of the powerful factors controlling the change of growing shoot apex into primordium of ear in rice plants.

The effect of special nutritive element on the formation of flower bud was proved fairly distinct. Nitrogen and phosphorus affected inversely with respect to the heading. When the plants wanted in nitrogen, their heading hastened, but the appearance of ear was delayed in the case of phosphorus deficiency.

Cutting off the first two or three leaf-blades or whole leaves from the seedlings had no effect on the growing of following leaves, but the loss of either one more leaf or leaf-brade resulted to delay the development of fifth and later leaves, consequently its heading was delayed several days. The fact suggests that a flower-promoting substance forms in the fourth leaf and later leaves and shows its action on initiation of flower bud, and also that the vegetative growth succeeds and developes a few more leaves before the tissue of stem tip receives the flower-promoting substance which has produced in the fifth leaf, when the substance is checked in the fourth leaf by cutting.

As far as these experiments are concerned, the influence of high temperature on the development of leaves on stem is insignificant. Under the combination of the two conditions, shortage of nitrogen and insufficient light have unexpectedly small number of leaves in the extra-early-maturing variety "Norin No. 11". So that, the minimum number of leaves on a stem in the variety could be decided less than five.

5. Studies on the root hairs in rice plant. Shin-ichiro KAWATA and Kuni ISHIHARA (Proc. Crop Sci. Soc. Japan, 27, 341-348, 1959)

The morphological studies on the development of the root hairs in rice plants which were grown in various conditions with reference to soils, were carried on in this experiment. It was found that the root hairs of the seedlings in the moist chamber were produced at about 1000 μ from the root tip and at about 4.1-13.0 mm in the paddy fields. Generally the root hairs were formed in the short cells of the epidermis of the root with polarity. When the soil of the paddy field was in the way of oxidation, the root hairs were found at farther distance from the root tip than in the reductive soil and furthermore they were numerous in number and longer in length. On the contrary, the formation of them was poor when the soil was reductive.

6. Morphological and physiological studies on nodule formation in leguminous crops. Part. III. The classification on the basis of the location and the origination in nodule

formation. Etuo KAMATA. (Proc. Crop Sci. Japan 26, 255-258, 1958)

In this experiment, 48 varieties of 17 species leguminous crops were used as materials. Each crops were planted in various experimental conditions, varying in amount of nitrogen supplied, light intensity and method of inoculation.

The external and internal observations were made on: 1) the location of nodule formation on root system, the nodule numbers and weight. 2) the site of nodule origin and the process of nodule formation with the histological methods.

The data obtained with the different varieties of the same species are not tabulated separately in this paper. The results obtained are as follows:

(1) The classification on the basis of the location of nodule formation on root systems.

I-A: The primary and lateral roots type: the nodule formation was found on the primary and all the lateral rootlets and the nodule's size and number were most large in the basal portion as compared with the apical portion in the same root.

I-B: The lateral roots type: the nodule formation occurred only on the lateral roots, even though the host plants were growing under widely different conditions, and the graduation of nodule formation in the same root are identical with I-A type.

(2) The classification on the basis of the site of the first infection cell in host tissues.

II-A: The outer layers in cortex type: *Phaseolus chrysanthos* SAVI., *Phaseolus vulgaris* L., *Glycine hispida* MAXIM., *Dolichos sesquipedalis* L., *Stizolobium Hassjoo* PIPER et TRACY., *Canavalia gladiata* DC., *Dolichos lablab* L., *Astragalus sinicus* L., *Lupinus luteus* L., *Lespedeza striata* (*Thunb*) H. & A., the infection is limited to the outer layers in cortex; endodermis, pricycles and central steles of theroot are not directly concerned in their formation other than in the production of the vascular system necessary in the later stage of nodule development. The site of penetration of nodule bacteria into host tissues and formation of the first infection cell have not correlated with the structure of central stele in roots, therefore, the location of nodules on root were at randam in the external view.

II-B: The inner layers in cortex type: *Pisum sativam* L., *Vicia faba* L., *Trifolium pratense* L., T. *repens* L., *Medicago sativa* L., *Vicia sativa* L., The nodules have originated by division of the parenchymatous cells in the inner layer of cortex in the host root, usually opposite protoxylem points. For this reason, the location of nodule formation on root were limited in underlying opposite the protexylem point.

II-C: The axil of lateral root type: *Arachis hypogaea* L.; the origin of nodules is in pericycle of cells adjacent and in juxtaposition to the protoxylem strand from which a rootlet had emerged. Therefore, all nodules were located in rootlet axil. In the peanut, this arrangement was conspicuous since the root system of this species consists of a central taproot, along which lateral roots arise in an orderly alignment of four vertical rows, owing to the tetrarck pattern of the xylem.

This classification have not correlated with the arc pattern of host roots, the apigeal or hypogeal on germinating stage of host plant and the numbers of the cell layers in cortex of host.

Part. IV. Factors determining the site of first infection cells in nodule formatian. Etuo KAMATA. (Proc. Crop Sci. Japan 26, 258-260, 1958)

In the previous paper, the author classified seventeen species of leguminous crops into three types according to the initial position of the nodule origination; namely, II-A type; nodule originated in the outer layers in cortex. II-B type; in the inner layers of cortex, and II-C type; the axil of lateral root.

The present experiment was carried out to clarify the relation between the site of first infection cells in different types and the physiological differentiation of host tissues as well as the fermentative characters of each nodule bacterium on the cultural mediums, especially, the minimum amount of carbohydrates required for the maximum growth of bacteria.

The results are as follows.

(1) The relation between the physiological differentiation or the gradients in the primary bodies of host tissues and the location of the first infection cell in host tissues.

The distributive pattern of reducing sugar, protein and some nitrogen compounds, and fatty matters in the cortex and the pericycle was the same in all plants investigated.

In general, the reactions of Fehling's solution and Ozazon method for reducing sugar, BENSLY's MILLON and NINHYDRIN reaction by BERG's for the protein and nitrogen compounds, Sudan Black Alcohol method for fatty matters were stronger at the inner layers of cortex, especially, at the opposite side of the protoxylem points with a progressive decrease towards the outer layer. These results indicated that the nodule origination in II-A type plants took place at where reaction of reducing sugar was the weakest in cortex, in II-B type plants it took place at where the sronger reacion of reducing sugar was shown and in II-C type plants at the pericycle cell showing the stronger reaction for reducing sugar.

(2) The relation between the sugar requirement of nodule bacteria and the location of the first infection cell in host tissues.

The nodule bacteria of different types differ with the minimum level of sugar in the cultural mediums.

The minimum concentrations of sugar in AO medium (M. R. N. RAGGIO, 1958) with manntol as carbon source for each bacteria are as follows, 0.01-0.2 gr/L for *Rhizobium Japonicum* (II-A type), 0.2-0.4 gr/L for R. *Leguminosarum* II-B type), and 0.1-0.2 gr/L for R. Sp (II-C type) respectively.

Part V. On the determination of location for nodule formation. Etuo KAMATA (Proc. Crop. Sci. Japan 27, 245-248, 1958)

The formation of nodules was limited generally at the basal portion of root or rootlet in leguminous crops even though growing under the different external conditions. These experiments were carried out to make clear the mechanism of the phenomenon above mentioned. 19 species, including 48 varities, of legminous crops were used as materials just like the previous experiments (KAMATA, 1958a).

All the crops were cultured with different levels of nitrogen supply, under different conditions of shading or on field. The distance from the base of root to nodule, the number of nodules in each level on root and the dry weight of nodules per plant were measured in the progress of growing of each crop. The development of nodules were observed at the basal portion of root or rootlet in all the cases examined, with only one exception, *Pisum Sativum* var. Beikoku Ohosaya.

The relation between the levels of both the differentiation and maturation of tissues in root and the formation of nodules was observed histologically with a serial cross-

sections and longitudinal sections of the whole primary root of *Glycine Max Mere* var. Norin. No. 1, *Pisum Sativum* var. Nihon Kinusaya and *Arachis Hypogaea* var. Southern Cross, and the following two cases were found. *Rizobium* penetrated into the host tissue at the portion of primary root, in which root hairs were growing, in soybeans and peas, but not bacterium was detected under the level of secondary root appearance on the primary root in peanuts.

The *Glycine Max Mere* var. Norin No. 1, *Pisum Sativum* var. Nihon Kinusaya and *Arachis Hypogaea var.* Southern Cross were cultured on sand under sterile condition. The primary roots of the seedlings, 12 cm in length, were divided into 5 pieces (ca. 2.0-2.5 cm), and the in lateral roots 3 pieces (ca. 2.6 cm). The contents of total sugar, reducing sugar and total nitrogen of the pieces obtained from equal level of roots were measured. Both the total and reducing sugar contents increased with aging of tissues, and the basal portion of root or rootlet had the highest concentration of sugar utilizable for the growth of nodule bacteria.

Part. VI. On the factors determining the location of nodule formation on root system in kidney bean and sword bean. Etuo KAMATA. (Proc. Crop Sci. Japan 27, 367–371, 1959)

In a previous paper (KAMATA 1958a), the author reported that eighteen leguminous crops might be classified into two groups which belonged to the following types; i.e. I-A type—nodules formed on both the primary and all the lateral roots, and I-B type—nodulation took place only on the lateral roots. This experiment was carried out to know the mechanism of nodule formation in I-B type using kidney bean (*Phaseolus vulgaris* L.) and sword bean (*Canavalia gladiata* D.C.) as materials.

By the histological study, it was clearly seen that the number of the cells in primary root, which were infected with penetration of Rhizobium were similar in both cases, I-B type (kidney bean and sword bean) and I-A type (soybean and peanut), even though the nodule scarcely formed afterwards on the primary root in the former. The weak staining with Heidenhain's iron-alum hematoxylin, when examined microscopically, proved no proliferation of nodule bacterium in the first infection cells of I-B type plants.

Cutting off a part of cotyledon from seed before sowing resulted to stop the development of nodule on a primary root in I-B type crops until flowering season. The nodule bacterium however, succeeded to grow, when it was cultured on Raggio's comsystematic extraction method from primary, secondary and tertiary roots of I-B type plants. The fact proved that the extract contained no inhibitor, which might be a toxic or promotive substance for the growth of bacterium in primary roots of I-B type plants.

In comparison of the amount of reducing sugar, non-reducing sugar and crude starch in root and rootlets of I-A type plant (soybean) and I-B type plants (kidney bean and sword bean) measured by SOMOGI's method, a complete parallelism was found between the concentration of available sugar, which was carbon source for growth of nodule bacterium in vitro, and the degree of nodule development. The amount of sugar available in the primary root of I-B type plants was lower and the amount of carbohydrate unavailable such as starch was higher as compared with those contained in other rootlets. Therefore, the nodule bacterium in the first infection cell might be inhibited to grow by the lack of available sugar in the primary roots in I-B type plants.

If the above mentioned supposition is appropriate, nodules must appear on primary root of I-B type crops, when unavailable carbohydrate in the root is changed into

available sugar.

The wilting treatment was thought to be one of the useful methods for changing carbohydrate to available sugar, and such results were obtained after treatments of wilting given three times during seedling stage as increase of available sugar, decrease of crude starch and the development of nodules on the primary roots in both the cases of kidney bean and sword bean.

7. **The effect of gibberellin on the spike initiation in wheat plants.** Aiya ISAIHARA (Proc. Crop Sci. Soc. Japan 27, 285–288, 1958)

Repeated sprayings of gibberellin solution, under the high temperature condition which was unfavorable for the progress of vernalization, stimulated the initiation of spikelet primordia in cold requiring wheats. It proves that gibberellin can replace the vernalization by low temperature. In such cases, before the spikelet initiation, the primordium differentiation in shoot apex was also promoted by the chemical. Moreover, there were some indications that the spikelet production in a young ear might be increased. In all gibberellin treated-plants, no discernible elongation of internodes was seen before spikelet initiation.

8. **Studies on the morphogenesis of crop plants under different growing conditions. Part. I. Changes of the leaf structure in *Phaseolus vulgaris*.** Koou YAMAZAKI (Proc. Crop Sci. Soc. Japan 27, 381–384, 1959)

A dwarf type of beans (*Phaseolus vulgaris*) was used for these experiments. They were planted in the field at different spacings (10×10 cm, 30×30 cm), and varying amount of fertilizer was applied. The structure of leaves grown under such conditions was investigated microscopically.

So far as these experiments were concerned, the difference of fertilizing made no effect on the structure of leaves, but the difference of spacing made a conspicuous effect, although fertilizing increased leaf size markedly. The leaves grown under closer spacing were reduced in thickness and had sparse arangement of mesophyll cells as well as broader areoles as compared with the ones grown under thinner spacing.

The development of these leaves was investigated anatomically and it was found that such differences occurred at the time when the leaf began to expand rapidly. Before this stage, when the cell division prevailed over the leaf, no difference was observed between the leaves grown under different conditions.

The structure of leaves grown under closer spacing coincides with the structure of so-called "shade leaf". Under closer spacing, the light intensity at the level of apical buds was 60∼70 per cent of that of thinner spacing. Therefore, the main factor responsible for the change in the leaf structure mentioned above may be light intensity. The effect of shading treatment on the leaf structure was the same as under the closer spacing, and moreover it was found that the shading at the expanding stage was most effective in causing the change of leaf structure.

9. **Nitrate tests as a diagnostic method of nutritional status of vegetable crops.** Tadayoshi SUGIYAMA and Kazuhiko TAKAHASHI. (Journ. Hort. Assoc. Japan 27, 161–170, 1958)

Nitrate nitrogen content in the vegetable crops and various factors affecting it were studied from the standpoint of tissue test or tissue analysis. Diphenylamine and

Bray's reagent were used for qualitative tests, and phenoldisulphonicacid method for quantitative tests of nitrates.

Ample storage of nitrates was found in the mature leaves of vegetables, especially in the petioles, and in the lower portions of stems, except onion group, in the leaves of which nitrates were scarcely found. Nitrate content was generally low in young leaves and upper portions of stems.

In some vegetables grown in sand culture in which nitrate levels had been kept constant, their nitrate content started low in young stage, reached its maximum in the middle age, then decreased as the plant grew older.

Nitrates could not be detected in the plants supplied with ammonium nitrogen only as nitrogen source in sand culture. In the field, nitrate content was lower in the plants applied with larger amount of ammonium sulfate than the ones applied with smaller amount or none of it in the early stages of growth. However, it was found that positive correlation had been established about one month or later after planting.

Diurnal change and change during storage up to for three days in room temperature in darkness or in difused light were not significant in nitrate content in the petioles of turnip leaves.

Controlled experiments in sand culture showed that changes in the nitrate level in the cultural solution were rapidly reflected in the nitrate content in the turnip leaves.

Field and laboratory works showed that the rapid tests by diphenylamine and Bray's reagent were simple and convenient for diagnosing nitrogen deficiency of vegetables in the field, while the tissue analysis method using phenoldisulphonicacid was accurate and reliable for diagnosing nitrogenous nutrition, when the portion and stage of crops of sampling were properly selected and definate standards for critical levels were available.

10. Effects of phosphorus and potassium supplied for the various stages of growth, on the yield and quality of seeds of Chinese cabbage in sand culture. Masatoshi IWATA and Yoshihiro EGUCHI (Journ. Hort. Assoc. Japan 27, 171–178, 1958)

Seed yields from plants supplied with phosphorus (15 ppm) from planting (late September) until the end of flowering (late April) were similar to those from control plants supplied with phosphorus throughout growth. Yields were lower when phosphorus was only supplied until the early flowering stage or until inflorescence extention began. In plants supplied with phosphorus until flower bud differentiation only, seed yield was reduced to two-fifth of that of the control. Seed phosphorus contents were also directly related to the time at which phosphorus supply was stopped. Rate of germination was not affected by phosphorus treatments, but the initial rate of radicle elongation was less with smaller seeds.

Plants supplied with 120 ppm potassium from planting until full bloom (late March), or until bolting, produced seed yields similar to those supplied with potassium at all stages of growth. When potassium was supplied only until flower bud differentiation, plants were severely potassium deficient, and seed yields were only one-third of those of the control. In treatments with no potassium in the early stages, growth became vigorous and seed yields were approximately normal if potassium supply began after mid-December. An application of potassium starting in mid-February, however, was too late for the plants to recover. There was no difference in the seed potassium concentration, percentage germination and initial rate of radicle elongation between the

potassium treatments.

11. **Effects of nitrogen supply for the various stages of growth on the yield and quality of potato in sand culture.** Masatoshi IWATA, Hiroshi TANAKA and Isamu MORITA (Journ. Hort. Assoc. Japan 28, 23–34, 1959)

Seed potatoes (Irish Cobbler) were planted late in March (1955) or early in April (1956), and plants were harvested early in July (1955) or middle in July (1956). The concentration of nitrogen in the solution was 140 ppm and other nutrient elements also supplied sufficiently.

The emergence of tubers in the minus nitrogen treatment was delayed five days compared with that of the control treatment in 1955. But the difference between both of treatments was only two days in 1956 experiment, in which larger seed potatoes were used (seed weight was 42-43 g in 1955, while about 110 g in 1956).

In both years, plants which were withheld nitrogen supply after late in June (almost ceasing stage of top growth) showed earlier yellowing of lower leaves, but their yields did not fall behind the control plants supplied with nitrogen throughout all stages of growth. But, when nitrogen supply was discontinued after late in May (beginning stage of tuber thickening), or early in June (vigorously growing stage of both top and tubers), yield decreased considerably. On supplying with nitrogen after early in May (completing stage of emergence), top growth caught up soon with that of the control, but tuber yield did not reach. And yield of plants supplied with nitrogen during the period between early in May and late in June, was almost the same as the yield of the control plants. Further, nitrogen supply being delayed till late in May, yield lowered considerably.

As to tuber quality, there were no distinct differences in dry matter percentage among the treatments. But, in ascorbic acid and total nitrogen, the earlier nitrogen supply was discontinued, or the later nitrogen supply was delayed, the lower were their contents.

In conclusion, the period most needed for nitrogen in this experiment, was between early in May (30-40 days after planting) and late in June (about 80 days after planting), and nitrogen supply from planting to emergence was also effective for yield increase. But nitrogen supply after late in June was not effective.

12. **A comparison of biological effects between thermal neutrons and X-rays on rice seeds.** Takane MATSUO, Hikoyuki YAMAGUCHI and Akihiko ANDO (Jap. Journ. Breed. 8, 37–45, 1958).

Rice varieties, irradiated seeds by X-rays with 250 kV. and 30 mA. through 1.0 mm.Al. filter and by thermal neutrons generated in column of the nuclear reactor at the Brookhaven National Lab. U.S.A. in 1957, were grown at B.N.L. under 30°C temperature and about 6,000 Lux. artificial light to test the variability of radio-sensitivity in infant stages in varieties and pre-treatments of seeds. Their R_1 and R_2 plants were also grown in the Exp. Farm of Tokyo University to see their fertilities and mutation rates.

The plant height and survivals in 20 days after irradiation and the number of grains per spike decreased with increasing doses, though the fluctuation of plant height was less in the case of neutrons irradiation than that of X-rays. The sensitivity of X-rays was higher in pre-soaked seeds than in dry seeds and also in Japonica varieties

than in Indica as seen in infant stages.

The fertility of R_1 plant decreased linearly with the increasing intensity of radiation and it seems to be caused by the structural changes of chromosomes due to irradiation from the view point of the distribution of fertility within R_1 plants.

The maximum frequency of chlorophyll mutation in R_2 generation was obtained by the irradiation of 20-30 kr. of X-rays and $4\text{-}5\times10^{13}$nth/cm^2 of neutrons, and the frequency of mutation was proportional to the dosage at the range of low doses, but at high doses the efficiency of mutation per unit dose decreased as compared with that at low doses. The spectrum of these induced mutations was similar in both radiations, though the late-sowing of irradiated seeds reduced the viridis-type mutants.

From the results, it was calculated that 1 r. of X-rays was equivalent to about 0.85×10^{8}nth/cm^2 for a reduction of spike fertility and in terms of the doses necessary to the induction of 5% frequency of chlorophyll mutation, 1 r. of X-rays was equivalent to aproximately 1.35×10^{9}nth/cm^2 of neutrons.

13. Studies on embryo culture in plants. Part 2. Embryo culture of interspecific hybrid in Oryza. Tetsuo NAKAJIMA and Hiroko MORISHIMA (Jap. Journ. Breed. 8, 105–110, 1958)

The authors crossed *O. minuta* PRESL. or *O.* sp. (*Paraguay*) to *O. sativa* L. In those cross combinations, the cross fertility was comparatively good, but the hybrid seeds were all imperfect grains. So, the weight of the hybrid grain was only one-tenth or one-twentieth of the normal *O. sativa* grain.

With investigation of embryogenesis in those hybrid seeds, the abnormal growth of scutellum and the abnormal development on the developmental axis of embryo were recognized, but the differentiation of the young shoot and primary root of embryo were nearly normal.

With sowing those hybrid seeds in Petri dish, the authors got few hybrid plants when female parents were diploid *O. sativa*, and no hybrid plants when female parents were tetraploid *O. sativa*. On the contrary, by means of artificial culture of those hybrid embroys in WHITE's agar medium, the authors got a number of hybrid plants when female parents were both diploid and tetraploid *O. sativa*. So, the authors could get the hybrid plants when female parents were tetraploid only by means of embryo culture.

The authors suggest that the technique of embryo culture is very effective to get the new hybrid plants in *Oryza*.

14. Weak expression of an awn-character mutated by X-ray. Hikoyuki YAMAGUCHI (Jap. Journ. Breed. 9, 28–32, 1959)

An X-ray induced mutant of 4-rowed winter barley produced awn of diverse types in a spike. This was more variable in the intensity of expression than the analogous-mutant *subcalcaroides* described by BANDLOW (1954). That is, this mutant made partly the same structural pattern, but gave different end product of development, as compared with *subcalcaroides*. As the mutant's spikes were divided into three kinds: n-, n→a- and a-types, X_3 progenies were classified, but no plant having spikes of n- and a-types was found in X and also in X_4 generations.

Although the seeds were selected from the plant having spikes of each type in X_3 generation, no change was observed in the appearance of abnormal plants in X_4.

The expression of mutated character intensified at the early differentiated part,

such as early heading spikes or the upper portion in a spike. Thus, it is probable that the spikelets of lateral row differentiate later in comparison with those of middle row at the same position. And it was estimated that the penetration of the mutated gene was about 70% to 80%.

In the cross with mother-type or hooded, the inheritance of mutant character was completely recessive.

From these results, it was concluded that the mutant character was induced by point mutation and its gene expression was affected by some environmental conditions, probably by temperature.

15. **Genetic studies on *Cosmos bipinnatus*. Part I. On the classification and inheritance of flower types.** Yoshihiko SAMATA (Jap. Journ. Breed. 8, 131–136 1958)

In this paper the author classified the flower-types of cosmos into the following four groups.—Single type (S type), Malformed type (M type), Double type (D type) and Malform-double mixed type (MD type), accompanied with genetic analyses of each of them. The important facts revealed by this study may be summed up as follows: (1) the Single and Non-petaled type are genetically fixed. (2) the Sub-petaled type (Ms type) seems to be the intermediate type between S and Np types. (3) the Tubular-petaled type (Mt type) and Vestigial-petaled type (Vt type) are different from Np type according to the number of modifiers. (4) Double flower-heads are usually represented by the genotype which has the single and the double factors in heterozygous condition. (5) Thed ifferent degrees of doubleness may be caused by the different number of modifiers added to a major gene of doubleness.

Part. 2. Further studies on the inheritance of Flower types. Yoshihiko SAMATA (Jap. Journ. Breed. 8, 261–268, 1959)

Further studies on the genetic constitutions of flower-types of cosmos were practiced and the following hypothesis has been proposed.

In the cosmos, two pairs of major genes and some modifiers affecting the shape of flower-heads can be assumed, one pair controlling the development of ray florets, and the other, affecting the development of disk florests. Symbols S-s, are given to the former and D-d to the latter. According to this assumption, the genotype for each flower-type can be represented as follows:

Single type: SSdd

Double type: SSDD or SSDd

Non-petaled type: ssdd, ssDd, or ssDD

Vestigial or Tubular-petaled type has the same major gene as Np, but differs from Np according to the number of modifiers.

Incompletely-doubled types have the same major gene as D, but differ from completely doubled type according to the number of modifiers.

It was found that each experimental result could be explained without contradiction if one permits the genotypes for each of the flower type.

16. **Heterosis of the starvation resistance in the silkworm immediately after ecdysis.** Hitoshi WATANABE (Journ. Sericul. Sci. Japan 28, 114–119, 1959)

Investigation was made on various inbred lines and their hybrids just after after entering into the third and the fifth larval stages to compare their starvation

resistance by median lethal time (LT-50) based on the starvation-mortality curve. The results obtained from the test indicated that there was no relation between sex and the resistance, and the latter was not affected by the body weight just before starvation, as well as by the maternal nutritive factors transmitted to the egg. The results also suggested that F_1 hybrids showed heterosis for the starvation resistance to a marked extent, as represented the value of heterosis from 20 percent to 50 percent of the mid-parent mean, and it was independent of the heterosis of the body weight.

17. **Synaptic transmission in the cockroach.** Teruo YAMASAKI and Toshio NARAHASHI (Nature, Lond. 182, 1805–1806, 1958)

Electrical phenomena associated with the synaptic transmission across the last abdominal ganglion of the American cockroach have been analyzed with special reference to the nature of the synaptic transmitter.

A presynaptic volley sets up excitatory postsynaptic potentials. When these reach a certain critical level, propagated action potentials are generated. An application of anticholinesterases causes a marked prolongation of synaptic after-discharge, the effect being attributable to the increase and prolongation of the excitatory postsynaptic potentials. The inactivation of cholinesterase after dosage with the anticholinesterases runs parallel with after-discharge. The threshold concentration of acetylcholine for discharging impulses can be lowered to 10^{-4} by eserinizing and desheathing the ganglion, although this value is still about one hundred times as high as for vertebrate ganglia and skeletal muscles.

A role of acetylcholine or acetylcholine-like substance as a synaptic transmitter is discussed.

18. **The effects of potassium and sodium ions on the resting and action potentials of the cockroach giant axon.** Teruo YAMASAKI and Toshio NARAHASHI (Journ. Ins. Physiol. 3, 146–158, 1959)

It has been recognized that the resting and action potentials from the giant axons of the squid are interpreted in terms of the concentration gradients of sodium and potassium ions between inside and outside of the membrane and the membrane permeabilities to these ions. However, it remains to be seen whether this theory of membrane potentials, the sodium theory, is also applicable to the cokroach axons.

The effects of varying the potassium and sodium concentrations in the bathing fluid on the resting and action potentials of the cockroach giant axons have been examined by means of intracellular microelectrodes. Plotting the resting potentials against the logarithms of the external potassium concentrations results in a straight line in the concentrations higher than about 20 mM. The decrease in the sodium concentration has no appreciable effect on the resting potential, but effectively depresses the action potential. Plotting the reversed potentials against the logarithms of the external sodium concentrations results in a straight line, which diverges only slightly from the theoretically expected straight line having a slope of 59 mV for a ten-fold change in sodium. The rate of rise of the action potential is decreased very effectively by sodium-defficient solution. It can be said safely that the over-all result constitutes strong evidence for the essential validity of the sodium theory in the cockroach giant axons.

19. **Eine neue Art von der Gattung *Oribotritia* Jacot (Acarina: Oribatei).** Jun-ichi

AOKI (Arach. Soc. East Asia 16, 18–20, 1953)

One new species of oribatid mites, belonging to the genus *Oribotritia*, was found in fallen leaves and humus from Kare-numa, Nikko, and was named *Oribotritia kishidai* n. sp. This new species differs from other species of the genus in (1) long, upright hairs on the hysterosoma, (2) arrangement of 3 pairs of propodosomal bristles, and (3) lack of bristle on the front part of analshield.

20. **Eine neue Gattung von *Carabodidae* aus der Insel Hachijo, Japan (Acarina : Oribatei).** Jun-ichi AOKI (Zool. Mag. 67, 390–392, 1958)

Mr. K. KANEKO (Tokyo Medical and Dental University) kindly brought me fallen leaves from Hachijo Island, in which the author found one interesting oribatid mite. It surely belongs to the family *Carabodidae*, but seems to differ from any other genera of the family, having double-structural lamellae and bristle-like curled sensilli. A new genus, *Diplobodes*, should be set up here for this animal. This new sepcies is named as *Diplobodes kanekoi* n. sp.

21. **On the seedage of cherries in Japan.** Fumio KITAMURA and Hiroshi INA (Journ. ap. Inst. Landscape Architects 22 [2], 1–4, 1958)

The authors picked up the folowing four species of cherries in Japan, "Yama-zakura (*Prunus Jamazakura* SIEB.)", "Somei-yoshino (*P. yedoensis* MATSUM.)", "Shi-dara-zakura (*P. Itozakura* SIEB.)" and "Jūnigatsu-zakura (one variety of *P. Lannesiana* WILSON)", and investigated their shapes of seeds, germination percentages, germination processes and growth of seedlings. The results obtained are summarized as follows:

1. The size and weight of seeds were in order from "Jūnigatsu-zakura", "Somei-"Yama-zakura", 24% of "Jūnigatsu-zakura", 22% of "Shidare-zakura" and 6% of

2. The germination percentage of fresh seeds sown in the frame showed 33% of "Yama-zakura", 24% of "Junigatsu-zakura", 22% of "Shidare-zakura" and 6% of "Somei-yoshino". In case of "Yama-zakura" sown in the field, or in the frame after being treated with 25% caustic soda solution, the percentage was about 10%, less than the former. As to "Yama-zakura" sown in autumn, the germination percentage of seeds which had been stored in wet sand, was better than that of fresh seeds, while the percentage of dry-stored seeds turned out low. In the plot where the storage was sown in the following year, the order of germination percentages of the four kinds of seeds had stored in the wet sand, was the same as fresh seeds and the percentage decreased to be less than ⅔. The dry-stored seeds failed to germinate totally.

3. The germination process of "Yama-zakura" is as follows: Under the proper condition, an embryo began to grow, a fissure came out in the stone and extended roots out of the stone. After two weeks or so, seed-leaves came off the stone and leaves came out on about 20th day. One week more, both seed-leaves and leaves opened completely and together with the straight stem, there appeared a perfect seedling.

4. As to the height of growing seedlings in their first year, "Somei-yoshino" came first and "Shidare-zakura", "Yama-zakura" and "Jūnigatsu-zakura" followed and the highest was found in the plot of sowing fresh seeds. In contrast with the height, the plot of sowing fresh seeds had the least branches which generally occurred to 10-20% of them.

22. **The forms of Japanese Zoysia in parks.** Fumio KITAMURA and Tomoo OZAWA (Journ. Jap. Inst. Landscape Architects 22 [3], 6–9, 1959)

In order to find out "the forms of Japanese Zoysia in parks", the authors made an investigation on "No-shiba (one variety of Japanese Zoysia) of the lawn in Shinjuku Gyoen Garden. In this place the following four as investigation plots were picked up:

A) Closed to the public but not taken care of
B) Closed to the public but well taken care of
C) Open to the public and properly trampled on
D) Open to the public and too much trampled on

and investigated "No-shiba", on eight items, in each plot twice a year, in spring and in autumn.

In the plot A, the number of leaves small; both the length of leaves and internodes were the longest; the fullness of internodes was poor; the number of branch-runners and roots was the smallest; and the growth of runners was the largest. In the plot B, the number of leaves was the largest; the length of leaves was short; the length of internodes was the shortest; the fullness of internodes was rich; branch-runners were the most numerous; roots were numerous; and the growth of runners was the poorest. In the plot C, the number of leaves was next to that of the plot B; the length of leaves was comparatively short; the damaged rate of leaves was low; the length of internodes was comparatively short; the fullness of internodes was the richest; branch-runners were comparatively numerous; roots were also numerous; and the growth of runners was comparatively poor. In the plot D, the number of leaves was small; the length of runners got poor; while leaves got numerous; branch-runners increased; and the nodes was comparatively short; the fullness of internodes was the poorest; branch-runners and roots were numerous; and the growth of runners was the poorest.

As a rule "No-shiba", when some physical pressure was put upon, showed the following feature: leaves got short; the length of internodes got short; and the growth of runners got poor; while leaves got numerous; branch-runners increased; and the fullness of internodes got rich.

To sum up, "No-shiba" in the plot B proved to be kept in the most suitable state for the lawn in parks because of its easy care and its beauty, and "No-shiba" in the plot C came next.

As a reference material, the authors counted the admission to the open plot of the lawn in Shinjuku Gyoen Garden. As a matter of course the number of people to the plot D was pretty bigger than that to the plot C, and it was in spring, not in autumn, the fact was that more people tended to visit the Garden.

23. **Studies on morphological and physiological characters of annual-garden-flowers. Part I. Influences of nitrogen nutrition on several annual-garden-flowers.** Fumio KITAMURA (Journ. Jap. Inst. Landscape Architects 22 [4], 12–15, 1959)

In order to find out the influences of nitrogen nutrition on morphological and physiological characters of annual-garden-flowers, "*Spray*", one variety of French marigold (*Tagetes patula* L.) and *Phlox Drummondii* HOOK. were grown in sand culture and the change of their form caused by want of nitrogen was investigated. The results obtained are summarized as follows:

1. The influences of nitrogen covered the whole growing period, and in case of using the nutrition solution contained nitrogen element, the growth of plants proved

to be better in every respect than the cultivated plants in the nutrition solution which had not contained nitrogen element in a certain growing period.

2. The influences caused by want of nitrogen were observed early in the number of branches and small leaves, and in the weight of leaves, small leaves and lateral buds. These defects, however, were recovered quickly by the additional supply of nitrogen. While the height of plants, the number of flowers and the flowering time were comparatively late in recovery.

3. The morphological influences by want of nitrogen mainly resulted from the unsatisfactory length of branches and the slow growth of plants. Compared with the plants cultivated in the nutrition solution contained nirogen element, these were smaller in size and more irregular in shape.

4. The influences of nitrogen nutrition on flowering were also great and a considerable amount of nitrogen was needed in the flowering period. Double-flowering of "*Spray*" owed much to nitrogen nutrition.

Judging from above mentioned, the author came to the conclusion that it is necessary for them to get the even supply of nitrogen nutrition for the whole processes of their growth and in order to bring about numerous flowers, long flowering period, bright leaf color and good shape, special attentions must be paid to this point.

II. Agricltural Chemistry

Abstract Nos. 24—68

24. **Studies on the effects of some physical conditions on the submerged mold culture. Part 1. The pellet type growth of asp. niger, and the effect of inoculum size on the type of growth.** Jyoji TAKAHASHI, Koichi YAMADA and Toshinobu ASAI (Journ. Agr. Chem. Soc. Japan, 32, 501, 1958)

In the shaking culture of molds, it is known that the mycelium may grow into the homogeneous, filamentous suspension (pulp type growth), or develop into the form of globose colonies (pellet type growth).

In this paper, the history of pellet type growth of Asp. niger NRRL-337, and the effect of the size of conidia inoculation on the type of growth, were investigated by shaking culture.

The results obtained were as follows.

1. Remarkable flocculences of germinating conidia were observed, from which the formation of pellet originates. That is, a pellet is formed by many hyphae germinated from different conidia.

2. The pellet type growth was always found in every size of inoculation (experiments were carried out in the range of 10^2—10^7 conidia/cc of medium). That is, such threshold number of conidia (about 10^5—10^6/cc) above which the mycelium develops into filamentous form in the case of Pen. chrysogenum as reported by Chain et al., could not be observed.

25. **Effect of the addition of pectinase on the pressing of grape fruits.** Yoshiyasu KOSANO, Jyoji TAKAHASHI and Koichi YAMADA (Journ. Fermentation 17, 265, 1959)

In making wine, it has been reported that the yields of grape juice and wine are

increased by the addition of pectinase to crushed grape fruits. Little is known, however, about the effect of the addition of pectinase and the pressing load on the pressing process of grape fruits.

From this point of view, the authors carried out an experiment from which the following results were obtained.

1) The yield of pressed juice (V) is given, under the constant load, by the following equation as a function of time (t).

$$V=\frac{\beta t}{\alpha+t}$$

hence, α, β: constant

Then,

$$\lim_{t\to\infty} V=\lim_{t\to\infty}\frac{\beta t}{\alpha+t}=\beta$$

Therefore, the maximum yield of pressed juice is given by β.

On the other hand, α controls the rate of pressed juice issue. That is, the rate of issue increases accordingly as the value of α decreases.

2) The effect of the addition of pectinase and pressing load on the values of α and β are shown in the following table.

addition of pectinase (+); added (−); free	pressing load (kg/cm^2)	α	β maximam yield of juice obtained from 1 kg of fruits
P (+)	0.1	1.056	761.16
P (+)	0.6	0.976	786.59
P (+)	1.2	0.944	811.56
P (−)	0.1	1.962	658.86
P (−)	0.6	1.905	711.90
P (−)	1.2	1.875	757.97

26. **Influence of the kind of salts and cream ripening on manufacture and keeping quality of butter.** Tomokichi TSUGO, Susumu KOYAMA, Kunio YAMAUCHI, Yoshikazu KOGA, Kenji SASAGO and Masahiro IWAIDA (Jap. Journ. Zootech. Sci. 29, 217–221, 1958)

Influence of the kind of salts on butter manufacturing process and on the quality of the product was studied by using flake salt (FS), specially refined salt (SRS), and pharmacopoeial salt (PS), with a small combined churn (capacity 30 lbs.). As the result, it was found that FS was more soluble than PS and had a tendency of distributing to butter more easily and uniformly during the working process. No difference was found, however, in salt distribution of finished products. The distribution of NaCl in SRS butter was similar to that in PS butter, but SRS butter tasted a little saltier than PS butter with the same salt concentration.

The butter manufactured from the cream ripened to 0.3—0.35% acidity with mixed starter (*Streptococcus lactis*: *Leuconostoc citrovorum*, 1 : 1) was superior in flavor to sweet cream butter. The results of TBA (thiobarbituric acid) reaction and flavor test showed no difference in fat oxidation between sour cream butter and sweet cream butter during storage for 14 weeks at 2°C. Thus, it was not found at all that sour cream butter was inferior in keeping quality to sweet cream butter.

27. **General properties of animal fibers. Part 2. Spectroscopic natures** Makoto KAMIYA

(Jap. Journ. Zootech. Sci. 30 41-45, 1959)

Spectrophotometric and spectrographic analyses were performed on the acid soluble and ashes of purified animal fibers of several species, and the following results were obtained. 1) Maximum absorbing was recognized at 284-285 mμ wave length in any wool and hair similar to human hair. Moreover, small peaks of light absorption were shown at 270-275 mμ.

2) The number of elements, the presence of which was certified in ashing matters, was sixteen or less. They were all metallic, except Si. Generally, white fibers had less contents of metals than colored ones.

3) Colors of animal fibers which had the same phase of light absorption spectra and similar components of metals were proved to be the same with one another and not related with species.

28. **Chemical properties of animal fibers. Part I. Content of nitrogen and sulfur** (Jap. Journ. Zootech. Sci. 29, 240-243, 1958)

The contents of nitrogen and sulfur in dry materials of purified animal fibers were analyzed and the following results were obtained.

1) The contents of nitrogen and sulfur were specific to each sepcies, except that the differences of soluble nitrogen contents were in significant among animal species.

2) No relatios were present between these contents and th color of animal fibers.

3) The differences of humin nitrogen content were significant among animal species.

4) The contents of total sulfur were equivalent to those of soluble sulfur, i.e. humin had no sulfur.

5) In comparison with the gravimetric method for the determination of sulfur, the volumetric method was found to be more suitable for microanalysis of this component of animal fibers.

Part 2. Amino acid components. Makoto KAMIYA (Jap. Journ. Zootech. Sci. 29, 277-282, 1958)

The amino acid components of purified animal fibers were analyzed by paper chromatography and the following results were obtained.

1) Seventeen kinds of amino acids were determined from this analysis, and the presence of tryptophan and lanthionine was suggested. Among these amino acids, cystine, glutamin acid, aspartic acid, leucines and arginine were abundant, but hystidine was always poor. Hydroxyproline was absent.

2) The differences of the amino acids contents, except glutamic acid, lysine and alanine, were significant between animal species. The situation was the same with NH_3-N contents determined by acid hydrolysis.

3) Comparison of the amino acid components, in which each component was calculated in grams to 100 g of cystine, has revealed the fact that Corriedale sheep wool and Angora-rabbit hair have similar amino acid components and so have tortoise-shell-cat hair and albino rat hair.

4) Cystine, glutamic acid, aspartic acid, leucines, and arginine are common, and main amino acids form the animal fiber protein.

5) The results obtained from paper chromatography of Corriedale-sheep wool were compared with those reported by other authors. This analytical method was

recognized to be applicable to the study of animal fibers.

29. General properties of animal fibers. Part I. Chemical composition Makoto KAMIYA Jap. Journ. zootech. Sci. 29, 214–216, 1958)

Fibers from the back of several animal species were analyzed with regard to 3 constituents and the following results were obtained.

1) As no correlations were present between the combined and the absorbed water contents of animal fibers, these two factors were considered to be different from each other. The differences in combined water contents among fibers of different animal species were insignificant.

2) The differences in contents of ether extracts and solids of purified hair and wool were both significant.

3) The contents of other three constituents mentioned above were more than 30% in wool and less than 1.0% in bristles. These facts are explained from the mechanism of hair grease secreation.

4) The contents of ashes in the purified solids of animal fibers were different in each species and related remarkably to the color of hair and wool.

30. Studies on milk coagulating enzymes. Part 8. Heterogeneity of α-casein and rennin coagulation. Part I. Tomokichi TSUGO and Kunio YAMAUCHI (Journ. Agr. Chem. Soc. Japan 33, 35, 1959)

Calcium-insoluble fraction (essentially identical fraction with α_R-casein of Long et al) and calcium-soluble fraction (crude κ-casein fraction of Long et al) were separated from Warner's α-casein and it was demonstrated that the mobility of the former was not affected by rennin but that of the latter was decreased after rennin action by means of Tiselius electrophoresis in phosphate NaCl buffer (pH 7.3; μ=0.1). It was further confirmed by electrophoretic experiment on mixtures of both fractions ($\alpha_R : \kappa =$ 4 : 1 or 2 : 1) treated with rennin separately. The disagreement of patterns between rennin-treated α-casein and mixture of two fractions treated with rennin separately was pointed out. It suggests that in unseparated α-casein interaction between both fractions is retained even after the rennin action.

Part 9. Heterogeneity of α-casein and rennin coagulation. Part II. Kunio YAMAUCHI and Tomokichi TSUGO (Journ. Agr. Chem. Soc. Japan 33, 37, 1959)

Calcium insoluble fraction (α_R-fraction) and calcium soluble fraction (crude κ-fraction) were separated from Warner's α-casein and rennin actions on both fractions were compared with each other. The former was coagulated with adding $CaCl_2$ (0.05 M), while the latter remained clear solution. After rennin action κ-fraction became clottable in the presence of $CaCl_2$ at 35°C but not at 0°C. Stabilizing action of the latter for the former was lost by rennin. κ-Fraction liberated non protein nitrogen (12% trichloroacetic acid soluble nitrogen) specifically (about 8% of total nitrogen) by rennin, while α_R-fraction was scarcely decomposed by it. κ-Fraction became opalescent in calcium free solution (0.085 M NaCl) by rennin and this opalescence was increased by heating (80°C, 5 minutes). α_R-Fraction showed no change by rennin. κ-Fraction became irreversibly denaturable by urea (6.66 M) after rennin action; the precipitate appeared in rennin treated κ-fraction after removal of urea by dialysis. It is assumed that rennin action is specific for κ-casein and that κ-casein transforms

into the structural configuration which is easily denatured by heating or urea treatment.

31. **Studies on the hydrolytic action of rennin on casein.** Umeo YOSHINO (Nippon Nogei-Kagaku Kaishi, 32, 736, 1958)

The proteolytic action of rennin on casein was studied. Crystalline rennin hydrolysed more casein than commercial rennet (Hansen's Rennet Tablet) at pH 6.0. Commercial rennet was thought to contain pepsin because it produced more hydrolysis products than crystalline rennin at pH 2.0.

The results of paper chromatography on 12% trichloroacetic acid soluble fraction produced from casein by rennin action showed that no free amino acid was liberated.

2% trichloroacetic acid soluble fraction produced from casein by rennin action was hydrolysed with hydrochloric acid and was analysed for amino acid by paper chromatography. The results showed that all kinds of the amino acids except tyrosine which were contained in casein, were detected in the soluble fraction. Absence of tyrosine in soluble fraction was also shown by tyrosine determination with phenol reagent, that is, when nitrogen of soluble fraction was amounted to 6% of total nitrogen, tyrosine in soluble fraction was equal to only 0.8% of total tyrosine in casein. By the action of trypsin (at pH 8.0), papain (at pH 7.5) or pepsin (at pH 2.0) the percentage of nitrogen and tyrosine moved to soluble fraction were parallel. Pepsin at pH 6.0, however, produced soluble fraction in which little tyrosine was contained.

In 2% trichloroacetic acid soluble fraction after ninety minutes' rennin action the increase of the relative amount of aspartic acid compared with that after fifteen minutes' when milk had coagulated was observed.

32. **Paperelectroretic studies on whey proteins.** Rinjiro SASAKI and Umeo YOSHINO (Nippon Nogei-Kagaku Kaishi 32, 733, 1998)

Paperelectrophoresis was applied for separation of whey proteins and hydrolysis products of casein by rennin.

The fractionated whey proteins on the strip of filter paper were detected by staining the paper strip with bromophenolblue and the amount of dye combined by separated proteins on paper was measured by dye elution method or direct scanning method. The elusion or direct scanning curves were compared with the pattern obtained with the moving boundary method and gave good agreement. β-lactoglobulin and α-lactalbumin were separated well but globulins were not fractionated clearly each other.

Whey from mastitis milk was studied by paperelectrophoresis and a fraction which migrated towards anode faster than β-lactoglobulin peak was detected. This fraction is thought to be serum albumin which usually exists in normal milk in small quantity. In mastitis milk, a kind of abnormal milk, it increased in amount and was detected on paper.

Casein solution was hydrolysed with crystalline rennin and an intact casein was precipitated by adding trichloroacetic acid to reaction mixture. 2% Trichloroacetic acid soluble fraction was concentrated in vacuo and examined by paperelectrophoresis at pH 7.2 with phosphate buffer. Two fractions were observed on paper. Each fraction was extracted with hot water and hydrolysed by hydrochloric acid. The results of the amino acid analysis by paper chromatography showed that the fraction which migrated to anode faster, contained more aspartic acid than the other.

33. **Studies on the action of rennet on whey proteins.** Umeo YOSHINO (Nippon Nogei Kagaku Kaishi 33, 487 1959)

The action of rennet on β-lactoglobulin was examined. An amount of rennet enough to coagulate milk in cheese making was used throughout this experiment.

The inhomogeneity was observed in the β-lactoglobulin peak of the electrophoretic patterns of rennet whey and the albumin fraction from rennet whey at the pH below 7.5, but was not observed at the pH above 8.1 and the pH 4.8. The β-lactoglobulin peaks of acid whey and albumin fraction from acid whey were homogeneous at the all pH ranges examined.

From the results of electrophoretic analyses and the determination of phosphorous content, the inhomogeneity of the β-lactoglobulin peak of rennet whey and the albumin fraction from it was supposed to be due to the hydrolysis product from casein with rennet. The concomitant substance was removed from α-lactalbumin in the β-lactoglobulin crystallization process and electrophoretically homogeneous β-lactoglobulin is crystallizable from rennet whey.

The inhomogeneity of the β-lactolobulin peak was not observed in the electrophoretic pattern of albumin fraction from rennet whey of heated milk (above 80°C foe 30 minutes). The hydrolysis product of casein with rennet was thought to be salted out with heat denatured globulins and casein.

No significant changes in viscosity, ultraviolet absorption spectrum, electrophoretic patterns, solubility in salt solution and formol titration value were observed in rennet-treated crystalline β-lactoglobulin solution. The appearance of the sulfhydryl groups in β-lactoglobulin solution with rennet action was not observed either.

34. **The effect of rennet on lactic acid bacteria in cheese ripening.** Umeo YOSHINO (Nippon Nogei-Kagaku Kaishi 33, 142, 1959)

The effects of rennet on the acid production and proteolysis of milk protein by lactic acid bacteria (Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Streptococcus cremoris, Streptococcus lactis and Streptococcus thermophilus) inoculated in milk were investigated.

The results indicate that the acid production by most of the lactic acid bacteria examined was depressed by the addition of rennet to milk, and none of the test organisms was stimulated to produce acid by rennet. The apparent inhibitory effect of rennet is due to the fact that in spite of the stimulatory ability of the hydrolyzed products of casein by rennet, casein is changed to paracasein which is found to be less available to lactic acid bacteria.

The increased proteolysis by lactic acid bacteria was observed in milk subjected to rennet action.

The experiment on the effect of pepsin on lactic acid bacteria gave the similar results as in the case of rennet.

35. **Studies on the effect of homogenizing casein solutions on the properties of casein.** Rinjiro SASAKI & Masahiro IWAIDA (Journ. Agr. Chem. Soc. Japan 33, 68-71, 1959)

The authors reported previously that when milk was processed under homogenization procedure the spherical form of casein was damaged. It is known that in milk itself casein exists in the form of micelles with calcium phosphate, the sizes of which ranging from 40 to 280 mμ.

In these experiments many kinds of casein solutions were prepared, such as 3% Na-caseinate solution, 3% Ca-caseinate solution, 3% casein chloride solution and so on. Each solution was homogenized at 2000, 3000, and 5000 lb. p.s.i. in a Gaulin homogenizer, and was subjected to the measurements of viscosity, tyrosine value, sulfhydryl group and peptic digestability, which were compared with the data previously obtained with calcium-phospho-caseinate.

From these results it became clear that effect of homogenization was largest when casein was homogenized in the shape of calcium-phospho-caseinate, secondly large with Ca- and Na-caseinate solutions and was the smallest with casein chloride solutions. No significant change was observed between Ca- and Na-caseinate solutions.

36. **Studies on the effect of homogenization on the properties of milk caseins.** Rinjiro SASAKI & Masahiro IWAIDA (Journ. Agr. Chem. Soc. Japan 32, 752–756, 1958)

It was already investigated by means of electron microscope that the spherical form of casein particles was damaged when milk was processed under homogenization procedure. The authors want to analyze more clearly the changes of the properties of casein caused by homogenization.

Fresh unpasteurized mixed herd milk was homogenized at 0, 1500+500 and 2500+500 lb. p.si. (two stage) in a Cherry-Burrel homogenizer. Purified whole casein was prepared from these three divisions of casein by acidfication, each of which was dissolved in sodium hydroxide to give a 3% solution of pH 6.8, and was submitted to the measurements of viscosity, tyrosine value, formol titration value and sulfhydryl group. The data indicate that a pronounced decrease (about 50%) occurs in the viscosity of homogenized casein, while the values of tyrosine, formol titration and sulfhydryl group increase a little. Peptic digestion was carried out with HCl solutions of the above mentioned three divisions of casein. It is commonly known that in the peptic digestion of the whole casein there appears a viscosity peak, which is attributed to the coagulation of casein particles. Regarding the homogenized milk casein, the peak is a little delayed and is much lower than the unhomogenized. No remarkable change is observed in the electrophoretic patterns.

37. **On the influence of homogenization on the property of milk. Part 2. Influence of homogenization on the proteins of milk.** (Jap. Journ. Zootech. Sci. 29, 287–289, 1958)

To study the influence of homogenization on the protein of milk, the electrophoretic patterns of skim milk, acid whey, acid casein and rennet casein which were prepared from homogenized and unhomogenized milk, were examined, respectively.

Fat globules were disintegrated to small particles by the homogenizing procedure. On account of the increase of their surface area, fat particles absorbed whey protein on them. This fact was confirmed from the electrophoretic pattern of milk casein and its whey.

In the electrophoretic pattern of rennet casein, the peak of α-casein of homogenized milk appeared more heterogenous than that of unhomogenizd milk. This change of α-casein must be confirmed by further, more detailed experiments.

38. **On the effect of a compound feedstuff containing urea upon milk production.** Rinjiro SASAKI (Jap. Journ. Zootech. Sci. 29, 294–296, 1958)

Feeding experiments with milking cows were performed on the galactogeneous

effect of two sorts of compound feedstuffs containing 3% urea each. One of them consisted eleven different feedstuffs and the other, thirteen.

One experiment was conducted with nine cows and the other with ten. The experimental feeding procedure was the same as the average farmer employs and the compound feedstuff was washed with much water.

The results indicate that the compound feedstuff containing 3% urea has the same effect on the production of milk as the common feedstuff and that it is a safe feeding to be employed by the farmer in his practical feeding procedure.

39. Studies on manufacture of semisoft cheese ripened by *Penicillium Caseicolum*, Tomokichi TSUGO, Toshio HAYASHI and Hiroatsu MATSUOKA (Jap. Journ. Zootech. Sci. 29 [6]. 345–349, 1959)

By using *Penicillium Caseicolum* semisoft cheese was manufactured in the authors' experimental factory. The usual procedure of manufacturing Camembert cheese was employed with some modification in temperature and time so as to obtain semisoft card. As a control, Gouda-type hard cheese was also prepared. Chemical changes were compared during the ripening process with these two types of cheese.

1. The semisoft cheese, after 3 weeks' ripening, had a milder flavor than Camembert and a more excellent flavor than Gouda, and seemed to satisfy Japanese consumers.

2. Comparing the semisoft cheese with Gouda-type cheese, the former ripened much more rapidly than the latter, judging from the degree of proteolysis and intensity of flavor. The former, after 3 weeks' ripening, showed the some or higher ripening rate than the latter after 3 months' ripening.

3. In the semisoft cheese, after 3 weeks' ripening, 17 or more kind of amino acids were detected by two-dimensional paper chromatography. It was found that aspartic acid disappeared from the semisoft cheese at the later period of ripening process, but it did not from Gouda-type cheese.

40. On the rate of mutarotation of lactose and β-galactosidase action on lactose isomers. Rinjiro SASAKI and Kokichi TANIGUCHI (Journ. Agr. Chem. Soc. Japan 33, 111–116, 1959)

It is well known that α- and β-lactose differ in some physical and chemical properties. Accordingly, the authors wanted to analyze their enzymological properties. Intestinal mucosa and yeast β-galactosidase were used in this experiment.

It is unavoidable that these two lactose isomers interconvert each other in solution, finally forming equilibrium state. Firstly, the specific rotative powers of α-, β-lactose and lactose at equilibrium state were determined at different temperature, secondly the rate of interconversion between two isomers was erucidated at different pH and temperature. These values are summarized in tables. From the above results, it became clear that the ratio of α- and β-lactose at equilibrium state tends to decrease with increasing temperature and the rate of interconversion between the isomers shows a minimum in the range of 3.0-5.0.

The differences of β-galactosidase action on two isomers of lactose were studied at 25°C, pH 5.0 for 20 minutes when the rate of interconversions was rather small and had not to be taken into account. β-Galactosidase has greater affinity to α-lactose than to β-lactose.

41. On the synthesis of α- and β-lactose-1-phosphate. Rinjiro SASAKI and Kokichi

TANIGUCHI (Journ. Agr. Chem. Soc. Japan 33, 183–186, 1959)

Attempts to synthesize α- and β-lactose-1-phosphate are made in this paper. The α-isomer is prepared by reacting the acetobromolactose with silver diphenyl phosphate and β-lactose isomer with silver dibenzyl phosphate. The specific rotations of prepared α- and β-lactose-1-phosphate are $+73.3°$ and $24.8°$ as Ba salt, and observed hydrolysis constants of them at 37° in 1 N acid are 0.0017 and 0.0057, respectively.

42. **Enzymatic and microbiological studies on lactose-1-phosphate.** Kokichi TANIGUCHI (Journ. Agr. Chem. Soc. Japan 33, 230–233, 1959)

It has been found by the authors that the β-galactosidase had greater affinity to α-lactose than to β-lactose. But the interconversion between α- and β-lactose were unavoidable. So in this experiment lactose derivatives, α- and β-lactose-1-phosphate, which did not interconvert each other, were used as substitute. The results of the experiments are as follows.

i) β-Galactosidase did not act on both α- and β-lactose-1-phosphate, therefore the difference of enzymic action on lactose isomers could not be recognized.

ii) *Esch. coli*, *Aer. aergenes* and lactose fermented yeasts which had both phosphatase and β-galactosidase action, could utilize lactose-1-phosphate as carbon source. But lactic acid bacteria which were lacking in phosphatase action, could not utilize lactose-1-phosphate.

iii) It became clear that α- and β-lactose-1-phosphate had almost the same effects on the growth and O_2-uptake of *Esch. coli* and *Sacch. fragilis.*

43. **Asparagine test in relation with the nitrogen nutritional status of crop plants. Part 1. Rice.** Shingo MITSUI, Kikuo KUMAZAWA and Mahatim SINGH (Soil and Plant Food 4, 189–195, 1959)

Since asparagine in the rice leaves has been regarded as an indication of nitrogen nutrtional status of rice plants by some of the previous research workers, "Nitrate test" being inapplicable for this aquatic plant, the present experiment was planned to investigate the mechanism of asparagine appearance and the role of various factors, particularly light intensities and nitrogen levels, towards its appearance. On the basis of the findings of this experiment it is quite definite that increasing duration of darkness is mostly responsible for the appearance of asparagine. However, in general, high amount of asparagine appears in the case of high nitrogen level than the low nitrogen level. Regarding the mechanism of asparagine appearance, the experiment has thrown light that soil nitrogn is the precursor of asparagine in the leaves. At the same time, asparagine appearance is closely related to the protein decomposition in the early panicle formation stage. Nitrogen compounds are decomposed and redistributed as low molecular compounds appear in the sampled sap. Asparagine test may be used in general for the determination of top-dressing requirement.

44. **Dynamic studies on the nutrients uptake by crop plants. Part 18. Effect of metaboic inhibitors on the uptake of nutrients by rice root grown under different nutritions conditions.** Shingo MITSUI and Kikuo KUMAZAWA (Journ. Sci. Soil and Manure, Japan, 29, 187–192, 1958)

Rice plants were preliminarily grown in complete culture solution, or in solutions deficient in phosphorus or potassium for a period of about three weeks, subsequent to two months' complete culture.

With these plants nitrogen, phosphorus, potassium and water uptake was determined under complete, phosphorus or potassium deficient nutritions conditions, respectively as sociated with or without such metabolic inhibitors as sodium azide, 2,4-dinitrophenol or sodium fluoride.

The uptake of NH_4-N was primarily dependent on the coexisting phosphorus and potassium, decreasing with the lack of these elements.

On the contrary, the uptake of phosphorus was primarily dependent on the preliminarily P and K nutritions conditions. Particularly by phosphorus deficient the uptake of phosphorus was remarkably increased. Also, the susceptibility to metabolic inhibitors seemed to decrease with the preliminary addition of P and K i.e. completely nourished.

The uptake of potassium was simultaneously dependent on the K nutritions condition and on the coexisting phosphorus. By potassium deficiency the absorption of potassium increased, and decreased by the lack of coexisting phosphorus.

The uptake of water was depressed by the low P and K nutritions conditions, similar to the uptake of NH_4-N.

Part 19. Salt effect and salt dilution effect of sodium diphosphate, superphosphate of lime and fused magnesium phosphate on the fixation of phosphatic acid. Shingo MITSUI and Kiyoshi TENSHO (Journ. Sci. Soil and Manure, Japan, 29, 221–226, 1958)

10 g of Tanashi soil of volcanic ash origin was shaken with 100 mg P_2O_5 of the respective phosphates and 10 m.e. of various salts in 100 ml. water. A portion of the supernatant was repeatedly refreshed with the same ml of distilled water. The fixation and release of phosphoric acid was measured as related with co-existence and dilution of salts. The results obtained are discussed and summarized as follows.

1) Disodium phosphate. The increased fixation of phosphoric acid with the accompanied salts (salt effect) was released on the repeated dilution showing peaks of PO_4 concentration, as contrasted with steady decrease in no-salt treatment (salt dilution effect). The salt dilution effect showed insignificant difference between chloride and sufate. The difference among cations, however, was outstanding, increasing in the order $Na \gg K > Ca$ as reverse to the salt effect.

2) Superphosphate of Lime. According to the acidity and the preexistence of gypsum of this chemical fertilizer, both effects were less pronounced and considerably complicated as compared with (1).

3) Fused Magnesium Phosphate. In case of this water hardly soluble phosphatic fertilizer the salts addition preliminarily resulted in the solution of phosphoric acid followed by its fixation on the soil. Accordingly, the salt dilution effect was most remarkable among the three phosphates.

The whole results were discussed on the basis of pH and complicated insoluble salt formation and in relation to the actual manurial effect of such compound phosphatic fertilizers.

Part 20. Salt effect on phosphate (P^{32}) uptake by crop plants grown on soil medium. Shingo MITSUI and Kiyoshi TENSHO (Journ. Sci. Soil and Manure, Japan 29, 510–514, 1959)

In order to clarify salt effect on phosphate uptake by wheat grown on soil medium, five seedling experiments using P^{32} and one pot experiment were conducted.

The uptake of P^{32} by seedlings grown on Tanashi volcanic ash soil was increased

by the presence of neutral salts. The effects of neutral salts were distinctively observed in the following order.

Sulfate>chloride, K>Na, Mg, Ca>NH_4

The similar salt effect governed the phosphate uptake and yield of wheat grown in pots up to the period of maturity, although the difference due to these salts was not significant.

According to the same seedling experiments in which KCl was used as a salt, the above mentioned positive salt effect was found most predominantly at the soil pH of 6.0 and at the rate of 3 me added per 100 g soil. The effect decreased gradually as pH and salt amount differed from optimum pH and salt amount increased.

On other two soils, strongly acidic diluvial Mikatagahara soil and Tamagawa alluvial Mizonokuchi soil, the salt effect was negative, the balance of salt effect presumably being much more remarkable on the soil than on the plant roots.

45. **The uptake of strontium-90 and calcium by lowland and upland rice from soil, and their distribution in the plants.** Kiyoshi TENSHO, Ko-Ling YEH and Shingo MITSUI (Journ. Sci. Soil and Manure, Japan, 30, 83–89, 1959)

The pot experiments, using strontium 90 and Tanashi volcanic ash soil, of lowland and upland rice were conducted by three different ways of applying Sr-90: basic mixed application with the upper two-thirds part of the soil, basic surface application and top dressing at the young panicle formation stage (for lowland rice only). In these experiments, we studied the absorption of strontium 90 and calcium through the roots and their movement and distribution in the plants, with major emphasis on comparison between these two elements.

(1) The content of Ca and Sr-90 based on air dry basis is high in the stems and leaves, and extremely low in heads, particularly these of hulled rice. Both elements show uniform distribution in all parts of plants. That is to say, generally, the content of these two elements is highest in lower leaf-blades, followed by upper leaf-blades, stem and sheath, husk, rachis, hulled rice, and polished rice in the order mentioned.

(2) The ratio of Sr-90 to Ca, however, is not uniform in all parts of a plant. It was shown that this ratio is lower in the seed part, that is, husk and hulled rice, than in any other parts, irrespective of the kind of plants and the method of Sr-90 application. No distinct difference was noticed in this ratio among bran, hulled rice and polished rice. Considering that this ratio is unusually low in the lower leaves of lowland rice in the top dressing pot, we made it clear that redistribution of Sr in plants is difficult.

(3) The ratio of Sr-90 to Ca is slightly higher in plants for mixed application than in the exchangeable part of the upper two-thirds part of soil after harvesting, but the ratio is lower in plants than in the hot-HCl extractable part of the soil.

(4) The amount of Sr-90 absorbed into plants differs according to the method of applying Sr-90. In the case of lowland rice, this amount is largest in top dressing followed by surface and then mixed application. In the case of upland rice, on the other hand, Sr-90 absorption is remarkably low in the surface application. Sr-90 absorption rate was 0.82-1.66% for lowland rice, and 0.19-0.53% for upland rice.

(5) The ratio of Sr-90 to Ca depending upon the method of applying Sr-90, also showed a trend similar to the afore-said trend in Sr-90 absorption. In the mixed application, however, no striking difference was noticed between lowland and upland

rice.

(6) From these results, we have indirectly verified that the unusually high S.U. value of contamination of the grains of rice and wheat, which was shown in the survey of Sr-90 contamination of crops, is not mainly through the roots, but is attributable to direct contamination caused by radioactive fallout. Moreover, it is suggestive that the superficial development of active root system of lowland rice may contribute to the higher contamination of Sr-90 via roots as compared with upland crops.

46. **An evaluation on the methods for selecting auxotrophic mutants for Aspergillus fungi.** Teiichi FUKAMI (Journ. Gener. Appl. Microbiol. 4 [4], 216–222, 1958)

Of all methods for selecting auxotrophic mutants of filamentous fungi, two methods were newly divised. The screening effects of these methods were evaluated by a reconstruction test as well as by a pracical test. As a result, the permanganate method and the heating method devised by the author were proved to be as usefull as the sulfite method and the filtration method. Details of the procedures are described.

47. **Studies on 5'-phosphodiesterases in microorganisms. Part I. Formation of nucleoside-5'-nonophosphates from yeast ribonucleic acid by *penicillium citrinum*** Akira KUNINAKA, Shin-ichiro OTSUKA, Yasuo KOBAYASHI and Kin-ichiro SAKAGUCHI (Bull. Agr. Chem. Soc. Japan, 23 [3], 239–243, 1959)

During the investigations conducted on the mode of degradation of ribonucleic acid by microorganisms, it was found that one strain of *Penicillium* produces nucleoside-5'-monophosphates from ribonucleic acid. Identification of these nucleoside-5'-monophosphates was carried out by means of chromatography on anion exchange column, ultraviolet spectrophotometry, paper electrophoresis, and characteristic color reactions using orcinol, carbazole, and rosanilline. It is suggested that this strain contains 5'-phosphodiesterase (or 5'-phosphodiesterase system) which cleaves all of the 5'-phosphodiester linkages in ribonucleic acid thus giving rise to nucleoside-5'-monophosphates. It is a point of interests in this study to indicate the presence of 5'-phosphodesterase for the first time in a common microorganism, as 5'-phosphodiesterase has been recognized only in somewhat particular materials such sa snake venom.

48. **Metabolism of aromatic compounds by bacteria. Part 1. Gentisic acid oxidase and Protocatechuic acid oxidase *of Pseudomonas ovalis*** S-5. Sin-ichi SUGIYAMA, Keiji YANO, Hirosato TANAKA, Kazuo KOMAGATA and Kei ARIMA. (Journ. Gener. Appl. Microbiol. 4 [4], 223–240, 1958)

Gentisic acid oxidase was obtained as cell-free extract from the cells of *Pseudomonas ovalis* S-5 which had been induced by *m*-hydroxybenzoic acid. Its purification and investigation of its properties were carried out, and the facts that this enzyme was a new ring splitting enzyme and required ferrous ion as a sole co-factor, were demonstrated.

Fumarylpyruvic acid which is the product of the ring cleavage, was obtained as crystalline form, and identified. Maleylpyruvic acid which is initial intermediate was recognized spectrophotometrically, and the metabolic pathway of gentisic acid oxidation was established.

Protocatechuic acid oxidase which was contaminated in crude extract, is constitutive enzyme in the cells of *Ps. ovalis*, and was purified. As the results from investigation of its properties, this enzyme also requires ferrous ion as a co-factor, and produces

β-carboxymuconic acid.

In secondary induction procedure, the inductive formation of *m-hydroxybenzoic* and gentisic acid oxidizing enzymes by intact cells prepared from the stationary phase were the strongest. Oxygen was necessary to this enzyme formation, however, the addition of glucose as energy source was ineffective. And other factors which influence secondary induction procedure, were determined.

Part 2. *m*-Hydroxybenzoic acid hydroxylase A and B; 5-Dehydroshikimic acid, a precursor of Protocatebhuic acid; A new pathway from Salicylic acid to gentisic acid. Keiji YANO and Kei ARIMA. (Journ. Gener. Appl. Micobiol. 4 [4], 241–258, 1958)

m-Hydroxybenzoic acid hydroxylase A which catalizes *m*-hydroxybenzoic acid to protocatechuic acid was obtained as cell-free enzyme from the cells of *Ps. dacunhae* A-7-2 and R-10-2, and *m*-hydroxybenzoic acid hydroxylase B which catalyzes *m*-hydroxybenzoic acid to gentisic acid was obtained from the cells of *Ps.* sp. 15-1A and strain 23-1A, 42-1A. Each enzyme requires TPN, probably TPNH, as a cofactor.

The constitutive enzymatic pathway *via* protocatechuic acid in the cells of *Ps. ovalis* S-5, was demonstrated, and its activity is strongest at the staionary phase during the growth of the cells. Shikimic acid is converted to protocatechuic acid by the incubation with the cells and this pathway is not inhibited by the addition of streptomycin 10 M.

From the incubation mixture of *Ps. ovalis* S-5 with shikimic acid, 5-dehydroshikimic acid was crystallized which is produced from shikimic acid and converted to protocatechuic acid. Thus, 5-dehydroshikimic acid was recognized as a preursor to protocatechuic acid.

The pathway from *m*-hydroxybenzoic acid was examined among the one hundred and thirty-three strains isolated from the soil and sewages. It was concluded that one hundred and nine strains had protocatechuic acid path. And a new pathway from salicylic acid to gentisic acid was recognized in the cells of *Ps.* sp. 15-1A. 15-4A and 40-2A.

49. Hiochic acid, a new growth factor for Hiochi Bacteria. Part I. Distribution of Hiochic acid. Gakuzo TAMURA and Atsuo NAGURA (Journ. Agri. Chem. Soc. Japan 32, 701, 1958)

Evidence is presented for the existence of a new factor required absolutely for the growth of 'True Hiochi Bacteria' i.e., *Lact. homohiochii* and *Lact. heterohiochii.*

The Sake (Japanese rice wine) which is frequently spoiled by these bacteria contains this factor. It was not detected in liver extract, yeast extract, malt extract, tomato juice, whey and laboratory peptones, but was found to be accumulated in the culture broth of several microorganisms, such as *Aspergillus, Penicillium, Monilia, Willia, Saccharomyces* and *Lactobacillus.*

The factor is an organic acid since it is extracted with ethylacetate or chloroform from the culture broth at acidic pH. It is relatively stable to alkali, but unsable to acid. All of the compounds of known structure examined were completely inactive.

Part II. Isolation of Hiochic acid. Gakuzo TAMURA (Journ. Agr. Chem. Soc. Japan 32, 707, 1958)

By means of bioautography it was confirmed that the new growth factors for "True

Hiochi Bacteria" produced by several organisms in the broth were all the same.

From the broth in which *Aspergillus oryzae* strain A was grown, the factor was extracted with butanol and or ethylacetate after being acidified to pH 2.0. A highly purified syrupy material was isolated by the chromatographic technique using silica gel, and a powdered barium salt of factor was obtained.

When the barium salt was acetylated with acetylchloride or acetic anhydride, its activity for the growth of Hiochi bacteria disappeared completely. However, the activity was recovered after alkali-hydrolysis of the acetyl derivative, indicating the presence of hydroxyl group. No color was developed upon the addition of ferric chloride. The aldehyde and ketone group tests were all negative. The olefinic unsaturation tests were also negative. It is concluded, therefore, that the factor is a saturated hydroxy acid.

The barium salt of the factor supports the maximum growth of *Lact. heterohilchii* strain H-1 at the concentration of 0.4 μg per ml of the medium. It is proposed to designate the factor by the name 'Hiochic acid'.

Part III. Nutritional requirements of Hiochi Bacteria. Gakuzo TAMURA and Yahiko SUZUKI (Journ. Agr. Chem. Soc. Japan 32, 778, 1958)

Using synthetic medium supplemented with hiochic acid as the basal medium, the nutritional requirements of 'True Hiochi Bacteria', *Lact. heterohiochii* strains H-1, H-3, H-5, and H-11, were studied.

Vitamin omission test revealed that thiamin, riboflavin, nicotinic acid, pantothenic acid and folic acid were needed for the growth of the test organsms. Hiochi Bacteria could not grow in the media from which either Tween-80 or peptides from liver autorisate or casein entimatic digest were omitted. Purine and pyrimidine bases have a growth stmulative effect.

It is also confirmed that the growth rate of Hiochi Bacteria depends upon the quantity of hiochic acid which are supplemented to the medium.

Part IV. Determination of structure of Hiochic acid. Gakuzo TAMURA (Journ. Agr. Chem. Soc. Japan, 32, 783, 1958)

Hiochic acid was isolated in crystalline forms as quinine salt ($C_{26}H_{30}O_6N_2$, m.p. 137-8°C) or S-benzylthiuronium salt ($C_{14}H_{22}O_4N_2S$, m.p. 122-3°C). A distilled syrupy lactone of this acid was also obtained ($C_6H_{10}O_3$, b.p. 130-135°C/2 mm, b.p. 145-150°C/5 mm, $N^{19}=1.474$, $[\alpha]_D^{20}-19.9°$, pk=4.3 g). From the chemical reactions, it was evident that the acid was a β-hydroxy acid and had no adjacent hydroxyl group. The infrared spectra of the lactone showed clearly the presence of δ-lactone. Therefore, the lacton of hiochic acid is a saturated β-hydroxy-δ-lactone having a formula of $C_6H_{10}O_3$. The following four structure β-hydroxy-δ-caprolactone (I), β-hydroxy-γ-methyl-δ-valerolatcone (II), β-hydroxy-β-methyl-δ-valerolactone (III), and β-hydroxy-α-methyl-α-valerolactone (IV), are compatible with the observation described above. Among these, structure I seemed to be most probable in view of hte fact that the iodeform formation test was positive. The α,β-unsaturated acid resulted from natural hiochic acid showed a definite peak of absorption at 222 mμ (ϵ=9,880) while the synthetic parasorbic acid showed only an end absorption. This fact obviously showed that the former is different from parasorbic acid, therefore, the presumption of structure I for hiochic acid must be excluded.

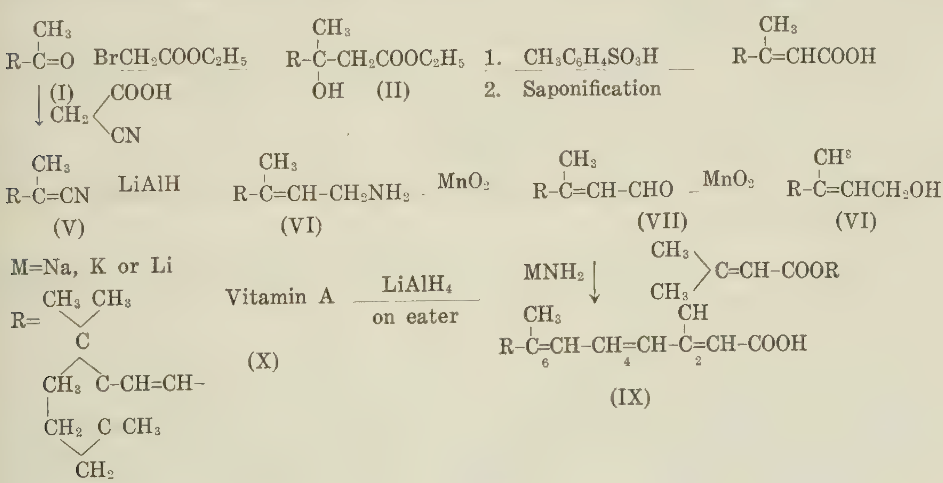
As the structure III is also compatible with most of the known characteristics of natural hiochic acid lactone, it was synthesized from-β-hydroxy-β-methyl-glutaric aldehyde by Tischenko reaction. The infrared spectra of the synthesized lactone (III) were entirely identical with natural hiochic acid lactone. The biological activity of the synthetic lactone was one half that of natural lactone. From these facts it is concluded that the lacone of hiochic acid is identical with structure III and hat the levoraory isomer of the lactone is biologically active.

50. **Cyanide insensitive terminal kespiratory system in *Aerobacter cloacas*** Shôji MIZUSHIMA, Michiko NAKANO and Kin-ichiro SAKAGUCHI (Journ. Biochemistry, 46, [3], 373, 1959)

1. *Aerobacter cloacae* isolated from soil was found to show a cynaide-insensitive growth adaptively.
2. The glucose oxidation by cyanide adapted cells was cyanide insensitive, whereas that by cyanide non-adapted cells was remarkably inhibited by cyanide.
3. The oxidation of DPNH and succinate by the cell-free extract preparation from both cyanide adapted and non-adapted cells, was cyanide insensitive. Almost all of the oxidase activity was located in the particulate fraction.
4. A cytochrome component which has absorption maxima at 561 and 530 mμ was detected in particulate fraction and was found to be reduced by the addition of DPNH, succinate and dithionite.
5. The physiological significance of this cyanide insensitive terminal oxidase system was discussed.

51. **Synthetic Studies on Vitamin A. Part 1. A Novel Synthesis of Vitamin A** Masanao MATSUI, Shigeru OKANO, Kyohei YAMASHITA, Masateru MIYANO, Seiichi KITAMURA, Akio KOBAYASHI, Tomiichi SATO and Ryuzo MIKAMI (Journ. Vitaminology 4, 178–189, 1958)

The authors found that the alkyl senecioate (VIII) condensed easily in the presence of alkali amide in liquid ammonia with β-ionylideneacetaldehyde (VII) and produced vitamin A acid (IX) in an excellent yield. The configuration of obtained (IX) was 2-trans when potassium amide was used, while in the case of sodamide or lithium



amide it was 2-cis. Thus four geometric isomers of vitamin A acid, i.e., all trans-; m.p. 182-3°, λ max 348 mμ, 46,300 (yield 60%), 2-cis-; 175-6°, 352 mμ 38,800)46,5% by $NaNH_2$, 15% by LNH_2), 6-cis-; 190-1°, 342 mμ 40,600 (60%), 2,6-dicis-; 133-5°, 346 mμ 31,050 (45%) were obtained from trans-(VII) (to which retro-(III) was converted by the acyl chloride procedure) and cis-(VII) (which was obtained by the chlorination of β-ionone acetate (II) and following dehydrochlorination). (VII) was also prepared through two routes.

These four vitamin A acids were converted to the methyl esters by two methods and lithium aluminium hydride reduction of them afforded a quantitative yield of vitamin A oils, all trans; λ max 325 mμ, ε 49,600 (was crystallized, m.p. 60-2°), neo; 328 mμ 41,800, 6-cis: 323 mμ, 31,500, 2,6-dicis; 325 mμ 31,400. Two acetates and four p-phenylazobenzoates were prepared.

Part II. The condensation of aldenhyde with senecioate (β, β-Dimethylacrylate) Masanao MATSUI, Shigeru OKANO, Kyohei YAMASHITA, Masateru MIYANO, Seiichi KITAMURA, Akio KOBAYASHI, Tomiichi SATO and Ryuzo MIKAMI (Journ. Vitaminology 4, 190–198, 1958)

In the presence of alkali amide the condensation of the aldehydes with senecioate is described.

The structures and steric configurations are determined by direct comparison with authentic samples, by isomerization with iodine and light or by ultraviolet absorption spectra. These results can be summerised as follows;

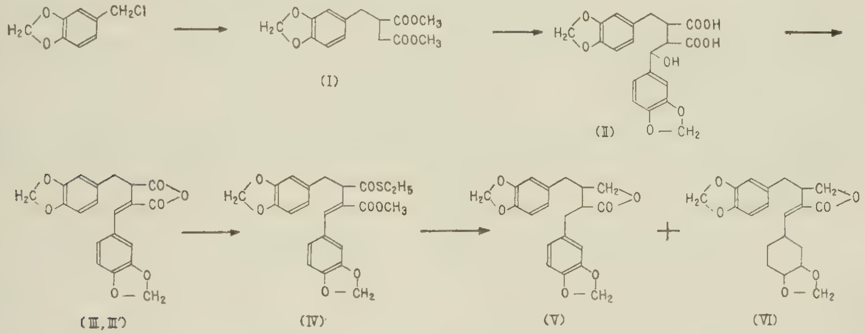
1) A condensation product of benzaldehyde and ethylsenecioate by means of sodamide, 3-methyl-5-phenylpentadienoic acid, has mp. 156° and is determined as 2-trans-4-cis acid. (λ max 307 ε max 28600).

   While potassiumamide is used as catalyst, the condensation product is identical with the isomerized acid from 2-trans-4-cis acid and they are determined as all trans acids (mp. 158° λ max 302 ε max 31000).
2) Condensation products of cinnamaldehyde and senecioate by means of sodamide and potassium amide are the same, (mp. 191°, λ max 330 ε max 45800) and have 2-trans-4-cis configuration, which are converted to all trans form (mp. 200° λ max) 327 ε max 55100) with iodine and light.
3) 3-Methyl-5-furyl-2,4-pentadienoic acid, obtained from furfural and senecioate by means of sodamide, is supposed to be 2-trans-4-cis acid (mp. 146.5° λ max 382 ε max 30300) and the other acid by means of potassium amide is deduced to be 2-cis-4-trans isomer (mp. 138° λ max 325 ε max 32400).
4) The yields of alkylidene senecioic acids are not so good as that of aromatic series. These acids can not be isomerized and they are believed to have all trans configurations.

52. **Studies on phenolic lactones. Part I. Synthesis of α-piperonylidene-β-piperonylbutyrolactone.** Kyohei YAMASHITA and Masanao MATSUI (Bull. Agr. Chem. Japan 22, 227, 1958)

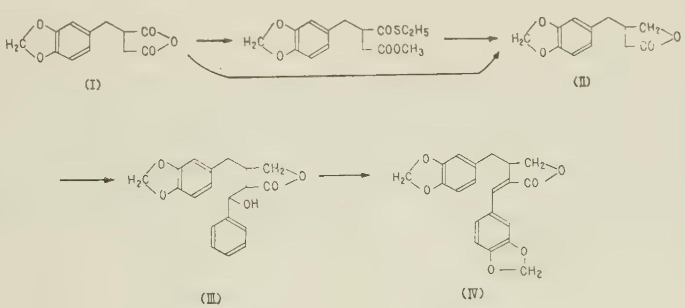
Hibalactone is the neutral component isolated from Chamaecyparis obtusa. The structure of hibalactone was concluded to be α-(3,4-methylenedioxybenzylidene)-β-(3,4-methylenedioxybenzyl)-butyrolactone. Because of its synergistic activities we attempted to synthesize it. Condensation of piperonal with diethyl piperonylsuccinate (I) afforded α - (3,4-methylenedioxyphenyl-hydroxymethyl) - β - (3,4-methylenedioxybenzyl) succinic

acid, m.p. 173-5°. Dehydration with *p*-toluenesulfonic acid or acetic anhydride gave two kinds of α-piperonylidene-β-piperonyl succinic anhydride (III, III') m.p. 167-8° and 115-7°, respectively. Reduction of α-piperonylidene-β-piperonyl succinic acid β-thioethyl α-methyl ester (IV) derived from (III') by Raney Ni catalyst followed by saponification and lactonization afforded α,β-dipiperonyl butyrolactone (hinokinin) (V), m.p. 107-8°, and a small amount of α-piperonylidene-β-piperonyl butyrolactone (VI), m.p. 154-5.5°. From the infrared and ultraviolet spectrum of synthesized unsaturated lactone it was assumed to be the geometrical isomer of racemic hibalactone and the authors designated it as isohibalactone.



Part II. A new synthetic method of Isohibalactone. Kyohei YAMASHITA and Masanao MATSUI (Bull. Agr. Chem. Soc. Japan 23, 230, 1959)

Isohibalactone the geometric isomer of hibalactone, was synthesized by the following route. Piperonyl succinic acid anhydride (I) was converted into thioethyl methyl ester (II) and was reduced to piperonyl butyrolactone (III) by Raney Ni catalyst. (II) was also prepared from piperonyl succinic anhydride by the reduction with amalgamated aluminum. Condensation of piperonal with the lactone (II) in the presence of potassium amide afforded α-(3,4-methylenedioxyphenyl-hydroxymethy)-β-(3,4-methylenedioxybenzyl)-butyrolactone (III), m.p. 151-2°. Dehydration of the hydroxylactone with *p*-toluenesulfonic acid gave isohibalactone (IV), m.p. 156-6.5°.



53. Syntheses and configurational elucidations of rotenoids. Part 5. Partial synthesis of rotenone and dihydrorotenone. Masateru MIYANO and Masanao MATSUI (Chem. Ber., 91,

2044, 1958)

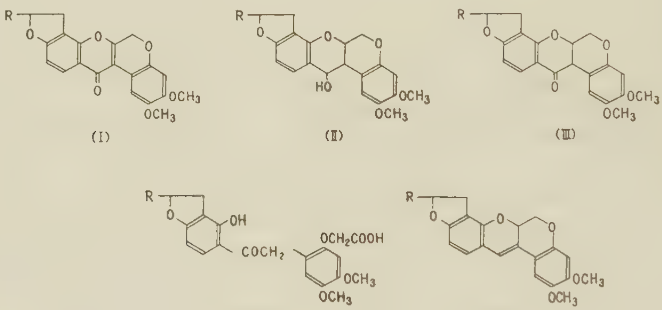
Reduction of dehydrorotenone (Ia) with sodium borohydride affords a new compound (IIa) named rotenol. Oppenauer oxidation of IIa results in colorless prisms melted at 128-129° which are found to be identical with mutarotenone. The synthetic mutarotenone when heated 5 minutes at 190° and recrystallized from hot ethanol affords colorless crystals melted at 162-3° which are identical with natural l-rotenone (IIIa) in all respects (IR-spectrum in Nujol paste, mixed melting point, etc.). Since derrisic acid (IVa) has already been converted into Ia by other authors, our result means the first partial synthesis of the natural rotenone from derrisic acid.

We obtained dihydrodehydrorotenone (Ib) from dihydroderrisic acid (IVb) by treatment with acetic anhydride and sodium acetate. IIa was then converted into dihydrorotenone (IIIb) through IIb. The synthetic dihydrorotenone is identical with the authentic specimen.

The constitution of rotenol (IIa) and dihydrorotenol (IIb) was further demonstrated by its dehydration with hydrochloric acid into desoxydehydro-compounds (Va, b).

A part of these results was already referred briefly in the previous paper (part IV, Bull. Agri. Chem. Soc. Japan 22, 128, 1958).

R represents $C\begin{smallmatrix}\nearrow CH_2 \\ \searrow CH_3\end{smallmatrix}$ in a series and $CH(CH_3)_2$ in b series.

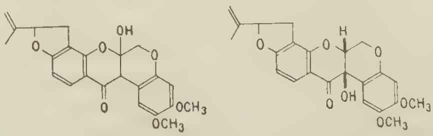


(I) (II) (III) (IV) (V)

Part 6. The structure and configuration of rotenolones. Masateru Miyano and Masanao Matsui (Bull. Agr. Chem. Soc. Japan 22, 335, 1958)

Hitherto the structural formula (I) has been claimed by other investigators for rotenolone-II, one of the air oxidation products of rotenone.

From the UV-spectrum together with its mode of formation we now present the structure and steric configuration II for rotenolone-II.



(I) (II)

Part 7. A synthesis of the natural rotenone from rotenolone-II, Further evidence of the structure of rotenolone-II, The steric configuration of the natural rotenone. Masateru MIYANO and Masanao MATSUI (Bull. Agr. Chem. Soc. Japan 22, 337, 1958)

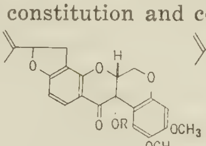
This paper is a short communication to the editors of Bull. Agr. Chem. Soc. Japan. Detailed descriptions are given in part VIII together with other new facts.

Part 8. Partial syntheses of the natural rotenone from rotenonolone-I and -II. Constitution and configuration of rotenolones, rotenolols and deguelinols. Masateru MIYANO and Masanao MATSUI (Chem. Ber., 92, 1438, 1959)

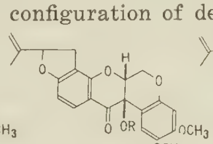
The constitution and configuration of rotenolone-I and -II, air-oxidation products of natural rotenone were determined to be I(R=H) and II(R=H) from the following facts. 1) UV-Spectrum of rotenolones clearly shows that they have no hydrogen atom at C-12, that is, the OH group is present in place of H. 2) Methylrotenolone-II (II,R=CH_3) rearranges to methylrotenolone-I (I,R=CH_3) in methanolic sulfuric acid and not vis á vis, therefore the latter is sterically more stable than the former. 3) Sodium borohydride reduction of rotenolone-I affords two glycols: rotenolol-Iα (III) and -Iβ (IV). An analogous reduction of ortenolone-II affords new glycols: rotenolol-IIα (V) and -IIβ (VI). 4) Rearrangement of rotenolol-Iα and -IIα with mineral acid gives colorless prisms which was found to be identical with natural rotenone (VII) in every respects. An analogcus rearrangement of rotenolol-Iβ resulted in a stereoisomer of natural rotenone. 5) Periodic acid oxidation of rotenolol-Iα and -IIα affords ketoaldehyde-A (IX), whereas the same oxidation of rotenolol-Iβ affords ketoaldehyde-B (X). Rotenolol-IIβ is not affected by periodic acid in an ordinary condition.

The mechanism of the rearrangement of rotenolol-Iα and -IIα to natural rotenone is discussed, from which the trans juncture of C-12,-13 in rotenone is concluded.

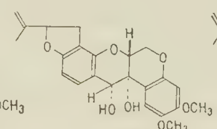
The constitution and configuration of deguelinol-I and -II are also discussed.



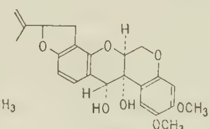
(I)



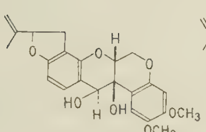
(II)



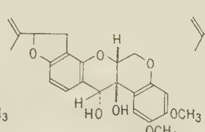
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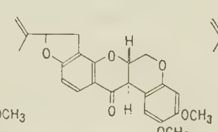
(IV)



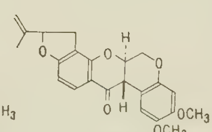
(V)



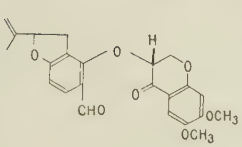
(VI)



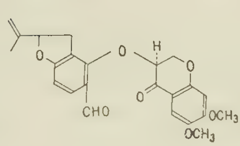
(VII)



(VIII)



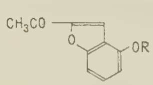
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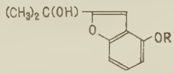
(X)

Part 9. A synthesis of roteol and hydroxyroteol. Masateru MIYANO and Masanao MATSUI (Bull. Agr. Chem. Soc. Japan 23, 141, 1959)

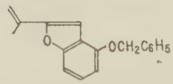
2-Acetyl-4-hydroxy-coumarone (I,R=H) when reacted with benzyl bromide in the presence of potassium carbonate afforded 2-acetyl-4-benzyloxy-coumarone (I,R= $CH_2C_6H_5$). Grignard reaction of I ($R=CH_2C_6H_5$) with methyl magnesium iodide formed two products: 2-(α-hydroxyisopropyl)-4-benzyloxy-coumarone (II, $R=CH_2C_6H_5$) and 2-isopropenyl-4-benzyloxy-coumarone (III) which can be well separated by chromatography on alumina. Catalytic hydrogenation of III in the presence of palladium-charcoal gives colorless oil which boils at 142-3°/10 mm and crystallized on standing spontaneously. After recrystallization from petroleum ether it melted at 59-60° and was found to be identical with roteol (IV, R=H), a degradation product of rotenone. An analogous hydrogeneration of II gave hydroxyroteol (IV, R=OH) of m.p. 160-161°.



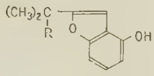
(I)



(II)



(III)



(IV)

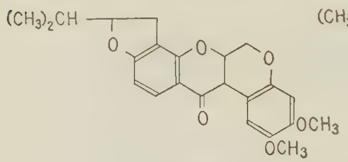
Part 10. Total synthesis of dihydrorotenone. Masateru MIYANO and Masanao MATSUI (Proc. Japan Academy 35, 175, 1959)

The first successful total synthesis of 1-dihydrorotenone is briefly described in this paper.

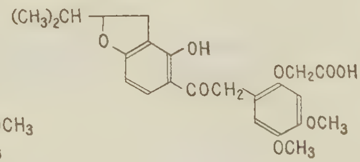
In part V of this series we reported a 3-step partial synthesis of 1-dihydrorotenone (I) from dihydroderrisic acid (II). Now we have succeeded in a synthesis of dihydroderrisic acid by Hoesch condensation of 1-dihydrotubanol (III) with a nitril (IV) following an ordinary saponification.

1-Dihydrotubanol has been known to be obtainable from roteol (V) in 5 steps, and the preparation of the latter from 2-acetyl-4-hydroxy-coumarone (VI) was already reported in part IX.

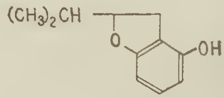
Since VI was prepared by other authors in 6 steps from resorcin, this report means 17-step total synthesis of 1-dihydrorotenone from resorcin.



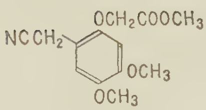
(I)



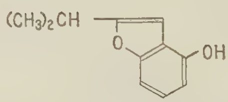
(II)



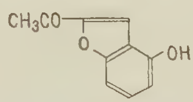
(III)



(IV)



(V)



(VI)

54. **A Model for A type humic acid.** Kyoichi KUMADA (Soil and Plant Food 4, 145, 1958)
Various organic compounds and soil humic acids were treated with boiling sulphuric acid, and the products alkali soluble and acid precipitable were compared with A type humic acids in respect to their absorption spectra and stabilities. It was found that the artificial humic acids prepared from anthraquinone or the pigment produced by *Penicillium islandicum* consisting of various hydroxyanthraquinones were very similar to the A type. On the other hand, the ones from B, and P type humc acid, terramycin, hydroquinone etc. were less similar, and from naphtazalin, glucose, cathechol etc. least. From these results, it was supposed that anthraquinone ring is at least one of the essential structural units in A type humic acids.

55. **The infrared absorption spectra of soil components.** Kyoichi KUMADA and Kazuyuki AIZAWA (Soil and Plant Food 4, 181, 1959)

Following the preceding paper, the authors examined the infrared absorption characteristics of soils. Soils were tentatively divided into the four components as follows:

The mixture of the soil and 0.5% sodium hydroxide, previously heated at 100°C for 30 minutes, was centrifuged (8000 r.p.m. for 30 minutes), and supernatant solution was dissolved in dilute alkali and ultrafiltered. The precipitate (U1) was obtained by acidifying filtrate. Black residue on the ultra-membrane was named U2. The surface black layer of the soil settled at the bottom of the centrifugal tube was named Z1, and the lower white or yellowish brown layer Z2. According to the infrared spectra, U1 corresponds to humic acid. U2 consists of organic matter and inorganic matter mainly composed of clay. Relative contents of these fractions differ from soil to soil. The organic matter is considered largely to be rather aliphatic and less soluble in alkali, but there contained a small amount of humic acid having higher degree of humification, compared with U1. It is presumed from the infrared spectra that dominant clay existing in U2 of the soil derived from volcanic ash belongs to allophane, and kaolin (?), and that of the soils of another origin to kaolin (probably kaolinite). In the order; U1→U2→Z1→Z2, the content of organic matter decreased remarkably. With the case of clay content the reverse happens, at the same time the absorption bands indicating the presence of quartz or silica became clear. Besides, the infrared absorption spectra were determined on some clay minerals, silica, alumina, iron oxide, aluminum, iron, and calcium humates etc.

56. **Studies on muscle proteins. Part 14. α-amino isobutyric acid as a constituent amino acid of protein.** Makoto KANDATSU and Keiichiro KIKUNO (Bull. Agr. Chem. Soc. Japan 22, 339–340, 1958)

Continuing our studies to clear up the presence of α-amino isobutyric acid in the muscle protein, we could now isolate this amino acid from both of the acid and pepsin hydrolysate of horse muscle proteins which were prepared from the hind leg.

Hydrolysate was converted to copper salt by the usual method, then the copper salt was fractionated with water and dry methanol, and the water soluble and methanol insoluble fraction were collected.

After removal of diamino and dicarboxylic acids, the filtrate was again treated to form copper salt, then the water soluble and methanol insoluble fraction was collected.

Monoamino monocarboxylic acid fraction was concentrated under vacuum and

treated by silica-gel chromatography with phenol: water (8:2). The eluded which only contained this amino acid, was shaken with ether to remove phenol and added with absolute ethanol to crystallize the amino acid.

By repetition of recrystallization with ethanol the white prismy crystalline was obtained.

The appearance of sublimation, results of elementary analysis are consistent with theoretical values. Melting point, elementary composition of picrate and picrolonate and also infra-red absorption spectrum of this amino acid are identical with a synthetic sample.

Accordingly, it is confirmed by us that α-amino isobutyric acid is one of the constituent amino acids of the muscle protein.

57. **On the determination of neutral amino acids by partition chromatography on silica gel.** Makoto KANDATSU and Hiroshi NAITO (Journ. Agr. Chem. Soc. Japan 33, 170–174, 1959)

The authors examined the partition chromatography whether it could apply to protein hydrolysate especially high in methionine content.

The procedure was essentially the same as that of TRISTRAM except the oxidative treatment of the protein hydrolysate which effects the separation of valine and methionine fractions, converting the latter to mehionine sulfone.

As for the quality of silica gel, which was prepared from the pure products, water content particularly the "structural water" of the gel, (i.e. weight loss of the gel by heating up to 600°) seemed to affect significantly the amino acid value.

Acetylated amino acid mixture was eluded with ethyl acetate, then developed by chloroform-n-butanol mixture in which the latter content was increased from 3 to 10% in the course of the chromatography. The recovery of amino acid was relatively constant (93-105%) except that of tyrosine in the oxidized mixture.

The partition chromatography was applied to the determination of several amino acids in casein and skeletal muscular protein of albino rat and it was shown that the amino acid values obtained were generally acceptable though the values of alanine, proline, tyrosine and methionine were variable.

The estimated value of valine and leucine isomrs (leucine plus isoleucine) agreed to those in literatures.

58. **Studies on reticulo-rumen digestion. Part VIII. On the vitamin B_{12}-like substances in the infusoria.** Makoto KANDATSU and Toshio OZAKI (Vitamins, 15, 559–563, 1958)

For the purpose of studying the biosynthesis of vitamin B_{12} by microbes in the rumen, the vitamin content of INFUSORIA belonging to CILIATA was measured by using Euglena gracilis var. bacillaris as a test organism.

The following results were obtained : (1) Vitamin B_{12} contents of INFUSORIA were about 40 to 100 μg per 100 g dry weight. (2) According to the results in the paper partion chromatography and bioautography the Euglena activity is not only due to vitamin B_{12} itself, but also vitamin B_{12}-like factors at least of two kinds.

Part IX. Distribution of nitrogen and cobalt in the rumen under fasting. Makoto KANDATSU, Bunpei MORI and Toshio OZAKI (Journ. Agr. Chem. Soc. Japan 33, 248–254, 1959)

The rumen content gathered through the fistula at the rumen of goat is divided into 6 parts, i.e., coarse fodder residue, infusoria, fine fodder residue, bacteria and

filtrate (infusoria and bacteria free fraction) by filtration and centrifuging.

The nitrogen and cobalt contents of each fraction are determined by usual methods titrimetrically and colorimetrically, respectively. The nitrogen content in the coarse fodder fraction decreases with the time course of fasting, but does not decrease in the total filtrate, being almost at the constant level. Among the total filtrate, remarkable change appears in the infusoria and bacteria fractions in such a way that at first the nitrogen content of both fractions deceases, but subsequently infusoria fraction increases after a while of increase of bacteria fraction and then both fractions decrease almost in the same way.

The cobalt content of each fraction changes as the nitrogen does on the whole. The number of infusoria per unit volume of total filtrate does not decrease against our expectation under fasting.

It is assumable from our results that cobalt has a more important role in the bacteria than in the infusoria among the microbes of the rumen.

Part X. On the vitamin B_{12}-like substances in the fasting goat. Makoto KANDATSU, Toshio OZAKI, and Bumpei MORI (Vitamins 16, 27–32, 1959)

To study the biosynthesis of vitamin B_{12} in the rumen, the rumen contents were fractionated into infusoria, bacteria, and infusoria-bacteria free fractions, and the vitamin B_{12} activities of each fraction were measured by using Euglena gracilis as a test organism.

The following results were obtained: (1) The vitamin B_{12} activity of total rumen contents was maximum, 4.2-5.2 μg per 100 g of fresh matter, 6-14 hours after feeding. (2) The vitamin B_{12} activities of infusoria and bacteria fractions were maximum, 0.73 μg and 1.53 μg per 100 g of fresh rumen contents, 14 hours after feeding, respectively. (3) The vitamin B_{12} activity of infusoria-bacteria free fraction was about 0.1 μg per 100 g of fresh rumen contents, and there was no remarkable change under fasting.

Part XI. On the Contribution of volatile fatty acids in the rumen. Makoto KANDATSU and Fumio KUMENO (Journ. Agr. Soc. Japan 33, 255–258, 1959)

Determinations of volatile fatty acids in the rumen of a goat fed on poor meadow hay and concentrate mixture were made. Mean levels of volatile fatty acids increased to peak value of 8-9 m.mol./100 ml. between 4-7th. after feeding. Then these decreased gradually to the level of 2-3 m.mol/100 ml. 48h. after feeding.

Variation of the molar percentage of individual volatile fatty acids was studied by partition chromatography. On feeding twice a day, there was a little variation in the molar percentage of individual acids. The range of that of acetic, propionic, and butyric acid was 67-73%, 16-22%, 7-13%, respectively.

The same result was gained on feeding once a day. The range of molar percentage of acetic, propionic, and butyricacid was 73-76%, 15-19%, 7-10%.

59. Studies on cecal digestion Part 2. Excretion of hard and soft feces and fecal composition in rabbits. Makoto KANDATSU, Ichiro YOSHIHARA, and TSUTOMU YOSHIDA. (Jap. Journ. Zootech. Sci. 29, 365–371, 1959)

The exertion time of hard and soft feces was investigated in rabbits together with the quantity of feces. Subsequently, quantitaive analysis of the feed given, hard and

soft feces, and cecal contents were carried out on crude protein, true protein, and crude fiber.

Silica, phosphorus (organic, inorganic, and lipid type), sulfur, magnesium, calcium, and iron contents were also determined in feces of both types.

The results obtained are as follows.

1. To prevent coprophagy effectively, steps were taken so that the mouth of a rabbit might not touch the floor wire-guaze of the cage.

2. The rabbit on the experimental ration voided soft feces which occupied about one-third of the total feces excerted or about one-quarter of the total amount on dry matter basis. When the ration was fed at about 10:30 A.M., soft feces began to be voided before 11:00 P.M., always accompanied with a small amount of feces of intermediate type between hard and soft. When the rabbit was kept without a wooden collar, only a little or no soft feces were found.

3. The soft feces contained much more moisture, crude protein, and true protein and remarkably less crude fiber than the hard feces. The composition of cecal contents was very similar to that of the soft feces.

4. In the soft feces and fecal contents, the proportion of true protein to crude protein was apparently smaller than that in the hard feces.

5. The proportion of hot-water-soluble ash to crude ash was much larger in the soft feces than that in the hard feces.

6. The phosphorus, sulfur, and magnesium contents of the soft feces were much higher than those of the hard feces. The content of alkalisoluble silica was reverse, while no remarkable difference was found in the iron content between both types of feces. The calcium content was lower in the soft feces than in the hard, but relative values calculated by using sand in the fodder as index was almost the same in feces of both types.

7. More inorganic phosphorus was contained in the feces than in the hard, but there was no difference in organic-phosphorus content between the two types of feces.

60. The transfer of ingesta from the rumen Part I. Sampling of rumen ingesta and estimation of the required length of the preliminary feeding period. Masao KAMETAKA and Hiroshi ITOH (Jap. Journ. Zootech. Sci. 29, 319–322, 1959)

In order to harvest a sample of uniformly mixed ingesta from the rumen, two methods are generally employed. In one method is used a permanent fistula and in the other surgical operation performed whenever sampling is required. In this paper, both methods were evaluated.

A permanent rumen fistula was affixed to a sheep weighing 30.5 kg. A large iron cannula (9.1 cm in outside diameter, 8.6 cm in inside diameter, and 5.3 cm long) was inserted through the fistula into the rumen. It was impossible, however, to leave this cannula attached to the sheep for more than one month.

The other method was applied to another sheep. When surgical operation was performed at the same site of the abdominal region to harvest the ingesta from the rumen, the sheep could stand the operation three times. When a different site was chosen in the abdominal region each time for operation, the operation could performed at least four times in the same animal.

It is necessary to determine the required length of the preliminary feeding period, a period from the beginning of feeding the ration on test to the time of sampling of

rumen ingesta. This determination was made by a tracer method with another sheep and goat. Barium sulphate containing radioactive isotope of sulphur, ^{35}S, was prepared by the author's method described in his previous report. $Ba^{35}SO_4$ suspended in a 5 per cent gelatin solution, was mixed with a ration of wild-grass hay chopped as uniformly as possible and given to two experimental animals. After the peroral administration, the collected feces were examined, after ashing, for radioactivity at different times. The appearance of radioactive material in the feces showed almost the same tendency in the two animals, the highest peak of excretion being found about the 28th hour after administration.

No radioactive material was detected from the feces of the sheep on test at the 6th hour after administration and those of the goat on test at the 12th hour. Moreover, a second peak of excretion may possibly be found about the 44th hour after administration in both trials. These results seem to be related to the mixing of barium sulphate in the rumen and/or the passage of this indicator through both the reticulum and the omasum.

From the results obtained in this experiment, it may be presumed that at least ten days are required for a preliminary feeding period, an interval between the beginning of feeding the ration on test and the time of sampling of rumen ingesta.

Part II. Estimation of the amount of ingesta transferred from the rumen. Masao Kametaka (Jap. Journ. Zootech. Sci. 29, 323–328, 1959)

A sheep and a goat were grazed on the meadow in the campus of the author's university, with receptacles for feces and urine attached to them. The amount of food intake was determined by the difference between the weights of the animals measured before and after grazing. The results obtained indicate that a sheep, weighing about 50 kg, had a food intake of 5.1 to 5.7 kg for 6 hours and a goat, weighing about 42 kg, of 2.1 to 2.9 kg for 4 hours.

A rumen fistula of large size was fixed to a goat so that samples of rumen ingesta might be collected from the four sacs of the rumen, i.e., the dorsal sac, ventral sac. ventral blind sac, and atrium, or anterior blind sac. The ingesta in the dorsal sac, closest to the fistula, were removed as much as possible by hand, weighed and mixed immediately. An aliquot of these ingesta was used as a sample for chemical analysis. The ingesta in the other sacs were then removed in order in a similar way. After sampling, all the ingesta removed were returned to the rumen in the reverse order. A wooden plug which can be separated into four equal parts, has been replaced by a plastic plug with a large orifice. The plastic plug is strong and convenient to use in the work described above, because it is not necessary to remove it from the fistula. Only about 40 minutes was enough to accomplish sampling with the plastic plug, as compared with about 1 hour required with the wooden plug. The ingesta in the dorsal sac had the smallest percentage of moisture content in all the trials. The entire quantity of the ingesta in the rumen of the goat averaged about 10 kg on a natural basis and about 1.3 kg on a dry-matter basis immediately after grazing for 4 hours.

The quantity of the ingesta transferred from the rumen in 22 hours, that is, the decrease in quantity of the ingesta in the rumen after 22 hours, was 32 to 34 per cent on a natural basis and 49 to 56 per cent on a dry-matter basis of the quantity of the ingesta present in the rumen immediately after grazing. It may be possible that part of the ingesta present in the rumen prior to grazing passed to the omasum and its

posterier portions of the alimentary canal while grazing, although no definite results have been obtained that such part of the ingesta was proportionate to the quantity of food intake. Moreover, it is presumed from the results obtained that one of the factors influencing the transfer of ingesta after grazing is the amount of ingesta present in the rumen, as measured on a natural basis rather than on a dry-matter basis.

61. Colorimetric determination of inorganic phosphorus in the presence of glucose-l-phosphate and adenosine triphosphate. Michinori NAKAMURA and Kenji MORI (Nature 182, 1441, 1958)

Whelan and Bailey (Biochem. J. **58**, 560, 1954) has reported that in the determination of inorganic phosphate by the method of Allen (Biochem. J., **34**, 858, 1940), the rate of hydrolysis of glucose-1-phosphate is reduced to one-seventh as compared with Allen's original method by halving the specified amount of the reducing agent in color development. As one of the present atuhors (M. N., Nippon Nogei-kagaku Kaishi (J. Agr. Chem. Soc. Japan), **24**, 1, 1950) developed, several years ago, a modification of Allen's method, in which sulfuric acid was used in place of perchloric acid and the amount of the amidol reagent was halved, and moreover, there has been no report pertaining to the effect of the amidol reagent, the experiments of Whelan and Bailey were re-examined. The results showed that the temperature at which color is developed is the most important factor. Thus, when color is developed at 33°C, the amount of the amidol reagent has some effect on the hydrolysis of glucose-1-phosphate and adenosine triphosphate; however, at 18°C, very little hydrolysis of these phosphate compounds occurs irrespective of the amount of the amidol reagent and the nature of the acid used (sulfuric acid or perchloric acid, each approximately at 0.5 N).

62. Determination of phosphorylase activity in the presence of β-amylase. Michinori NAKAMURA (Journ. Biochem., Japan 46, 799–806, 1959)

Detailed procedures are given for the quantitative determination of phosphorylase activity in the presence of potent β-amylase. This method is based on the finding that β-amylase is more sensitive to mercuric chloride than is phosphorylase. By correcting the observed phosphorylase activity in the presence of a suitable concentration of mercury for the recovery of added phosphorylase in the same digest, true phosphorylase activity is obtained. It was found by this method that crude extracts from sweet potatoes are very potent in phosphorylase activities.

63. Chemical studies on the autolysis (rigor mortis and reversal) of meats. Part 2. The comparison of glycolysis of meats. Rinjiro SASAKI and Masao FUJIMAKI (Journ. Agr. Chem. Soc. Japan, 32, 628–631, 1958)

Process of glycolysis after death was compared with pork, horse, and chicken meats by storage at 0-4° and determining glycogen and P compounds. Horse meat contained 2341 mg% glycogen immediately after slaughter, about 80% remaining after 7 days of aging. The glycogen content of pork was about 900 mg% and it decreased only to 20% after 1-day aging; the glycogen in chicken meat was nearly constant during aging, being lower in the order: pork, chicken, and horse meats.

Changes in inorganic P, released inorganic P after 7 min. hydrolysis, and glucose-1-phosphate indicated that post mortem changes in chicken meat had processed and finished in a short time after slaughter.

In horse and chicken meats, deamination of adenylic acid processed rapidly after slaughter, and the process of deamination was the same type as in the case of death in agony. It was considered that the aging process was different in these three kinds of meats.

Part 3. The colorimetric micromethod for the determination of glycogen and the existence of bound glycogen is meats. (1) Masao FUJIMAKI, Reiko SUZUKI, and Nobuhiko ARAKAWA (Journ. Agr. Chem. Soc. Japan, 32, 631–635, 1958)

Colorimetric glycogen determination of Kemp et al. was examined and modified to apply to meat glycogen. Though good results were obtained by the original method on horse meat or cattle liver, the method could not be applied to beef or pork owing to the presence of substances which interfere with coloration. The difficulty was avoided by treating meat with alkali and precipitating glycogen with ethanol prior to determination. Results obtained by the modified method coincided well with those by reducing sugar determination after hydrolysis of glycogen.

Variations in glycogen and so-called "combined glycogen" were determined on meats during aging at 0°. The percentage of combined glycogen to total carbohydrate in horse meat was 40-54 immediately after slaughter, which decreased to 27-28 after 2 days and to 15-21 after 4 days.

Part 4. Extraction and determination of myosin and actomyosin. Masao FUJIMAKI (Journ. Agr. Chem. Soc. Japan, 32, 689–695, 1958)

Nonprotein N in meats did not increase seriously during aging, except immediately after rigor mortis. Thus, the tenderness of meats was not dependent on the proteolysis. Methods of determining myosin, actin, actomyosin, sarcoplasmic protein, and stroma were discussed, examined, and the method of Hasselbach and Schneider was modified. Fractionation and determination of actomyosin were involved in the proposed method. The method was applied to the fractional determination of rabbit-meat proteins during aging.

Part 5. Changes of fibrillar and sarcoplasmic proteins during aging of meats. Masao FUJIMAKI and Yutaka NAKAJIMA (Journ. Agr. Chem. Soc. Japan, 32, 695–700, 1958)

Changes in contents of myosin, actin, actomyosin, globular protein, and stroma were estimated during aging of veal and rabbit muscle. Myosin and actin decreased rapidly, and actomyosin increased with onset of rigor mortis. But reversal and the tenderness of meats were not dependent on dissociation of actomyosin nor on the proteolysis of meats. Stoma increased on rigor mortis and decreased with reversal. Actomyosin was fractionated into 2 fractions, named actomyosin-S and -L, which represented the actomyosin obtained by a short time extraction with 0.6 M KCL, and that by a long time extraction with Weber-Edsall solution, respectively. Variations in these two fractions seemed to have some relation with rigor mortis and reversal. In meats immediately after death, the content of fibrillar protein was almost the same in many kinds of animals, but that of globular proteins was different from the kinds of animals. Meats with much globular proteins seemed to have higher water-binding capacity and higher stickness (binding capacity). Water-soluble protein seemed to receive little change during aging when examined by electrophoresis.

Part 6. Adenosinetriphosphatase activities of myosin and actomyosin in meats. Masao FUJIMAKI (Journ. Agr. Chem. Soc. Japan, 32, 770–774, 1958)

Conditions were examined for determining adenosinetriphosphatase (ATPase) activities in various protein fractions from meats, and the optimum pH was determined to be 6.8, 6.5 and 5.9-6.2, for myosin-ATPase, actomyosin-S-ATPase, and actomyosin-L-ATPase respectively. The actomyosin was activated by Ca and inhibited by Mg, the degree of inhibition by the latter being higher on actomyosin than on myosin. The ATPase activity was in the order: myosin > actomyosin-L > actomyosin-S, when compared with the same enzyme concentration in preparations from the same meat.

When myosin and actomyosin solutions were stored at 0° for two weeks, there occurred little variation in ATPase activity but much decrease in SH-group content. Thus, there seemed to be no parallelism between ATPase activity and SH content. But both ATPase activity and SH content decreased on addition of urea. ATPase of water and KCL extract from meats decreased during aging and it seemed not only due to quantity changes of myosin and actomyosin but also due to alterations of ATPase activities of these proteins.

Part 7. On the chemical changes of myosin B during aging of meats. Masao FUJIMAKI and Nobuhiko ARAKAWA (Bull. Agr. Chem. Soc. Japan, 22, 249–255, 1958)

In order to decide whether or not, the chemical properties of myosin B extracted, isolated, and prepared from rabbit, veal, cow, and horse meat have been altered during aging, experiments were carried out on salting out, viscosity, ATP-sensitivity, and the ATPase of myosin B solution.

1) Salting out experiments showed that there is no difference of myosin B to be observed in accordance with the kinds of animals tested. However, in the salting out procedure of AMS and AML solutions, the concentration of ammonium sulfate at the maximum precipitating region was found to be different. The concentration of ammonium sulfate in the salting out of myosin B was altered with the aging of meats; especially, in the case of AMS the maximum precipitating region seemed to approach that of myosin.

2) Among all the kinds of animal meats tested, intrinsic viscosity of AML was higher than that of AMS, and intrinsic viscosity of myosin B was altered with the aging of meats. Intrinsic viscosity of the myosin B solution seemed to be different according to the kind of meat, i.e., that of myosin B from rabbit high, while that obtained from cow meat was low.

3) The ATP-sensitivity of AML was higher than that of AMS. The ATP-sensitivity of AMS became very low as aging proceeded. From this result, it was considered that AMS turns to assume a high ratio of myosin in the constitution of actomyosin, and comes to resemble myosin during aging.

4) With the aging of meats, the optimum pH for ATPase of myosin B from rabbit and veal meat turned to the acidic side. At the neutral optimum pH, enzymatic activity showed a tendency to decrease during aging. Immediately after slaughter, the activity of ATPase of AMS became higher than that of AML, but the inverse relation was maintained during aging process.

Immediately after slaughter, the optimum pH for enzymatic activity of AMS from rabbit were found to be 7.3 and 10.2, activity at pH 10.2 being higher than that at pH 7.3. However no difference was observed at both of these pH values in ATPase

activity of AMS from veal immediately after slaughter.

Part 8. Influence of aging of meats upon hydration of meat proteins. Masao FUJIMAKI and Hiroko KURABAYASHI (Journ. Agr. Chem. Soc. Japan, 32, 775–778, 1958)

Water-binding capacity, which is considered to represent the degree of protein hydration, $(W\text{-}F)/W \times 100$ where W was total H_2O in meats and F the H_2O liberated by heating at 70°, was as follows; beef 65.4-67.4, pork 65.8-67.8, chicken 58.8-60.8, tuna 71.8-92.4, and cod 64.3-88.7%. The value varied with pH, the lowest value being observed at pH 5.3-5.5. Addition of ortho-, pyro-, tripoly-, or hexameta-phosphate to meats increased the water-binding capacity; ascorbic acid and its salts also increased the capacity to a lesser extent than phosphates did. Variations in the degree of hydration were followed during the aging of rabbit and horse meats. The variation was not only due to pH variations in meats, but to reactions of protein which might occur during the process.

Part 9. On combinations of muscular proteins with anionic and cationic dyes during aging of meats. Masao FUJIMAKI and Nobuhiko ARAKAWA (Journ. Agr. Chem. Soc. Japan, 32, 858–861, 1958)

Combining changes of actin and actomyosin with dye ions during meats were recognized by equilibrium dialysis method. The combining capacity of actomyosin-S with dye ion was larger than that of actomyosin-L. Different from most globular proteins, the combining capacity of actomyosin with methylene blue was larger than that with methyl orange.

The combining capacity of actomyosin-S with dye ion was maximum at two days after slaughter. Especially the combination with a cathionic dye, methylene blue was found to be prominent. The relation between this change and the water-combining capacity was considered. During aging of meats, the combining capacity of actomyosin-L and actin with methylene blue decreased. The combining capacity of actomyosin with dye ion seemed to be different from various meats.

Part 10. On the changes of myoglobin and its derivatives during the aging of meats. (1) Masao FUJIMAKI and Reiko OKURA (Journ. Agr. Chem. Soc. Japan, 33, 101–104, 1959)

Myoglobin from several kind of meats was determined colorimetrically. Changes of myoglobin and its derivatives during the aging of meats were studied from the results of absorption curves in water extractive solution of aged meats.

Part 11. On the changes of myoglobin and its derivatives during the aging of meats. (2) Masao FUJIMAKI and Masako HASEGAWA (Journ. Agr. Chem. Soc. Japan, 33, 104–110, 1958)

(1) Method of extraction of nitroso haem pigment from heated cured meats was studied.

(2) The content of nitroso haem pigment extracted from heated cured meats changed from the period of curing, and cured meats showed the maximum value from two to four days after curing.

(3) Effects of curing materials upon the fixation of meat color in cured meats and its mechanism were studied.

(4) Optimum condition for the fixation of meat color in hot curing, as used in the curing of canned corned beef was studied. Fixed meat color in hot curing seemed

to be slightly inferior to the one in normal (cold) curing from the determination of nitroso haem pigment.

Part 12. On the changes of free amino acids in meats during aging. Rinjiro SASAKI, Masao FUJIMAKI and Takehiko KAWANO (Journ. Agr. Chem. Soc. Japan, 33, 186–189, 1959)

Free amino acids in meats immediately after the slaughter of animals and during aging of meats were detected by paper partition chromatography. Results obtained are as follows.

(1) Free amino acids in meats (pork, chicken, carp, bonito and tunny meats) immediately after the slaughter of animals were glycine, alanine and glutamic acid (except carp meat).

(2) The contents and kinds of free amino acids in meats seemed to be more in pork, bonito and tunny meat than in chicken and carp meat.

(3) Free amino acids increased during aging of meats, and amino acids of threonine, leucine, isoleucine and phenyalanine which were not found immediately after the slaughter, occurred during aging.

Part 13. On yellow colored spot by ninhydrin in the two dimentional paper partition chromatography of meat extracts. Masao FUJIMAKI, Nabuhiko ARAKAWA and Miyoko WATANABE (Journ. Agr. Chem. Soc. Japan, 33, 190–193, 1958)

The spot with fluorescence under ultraviolet light, and yellow color by ninhydrin treatment, in the two dimentional paper chromatogram of alcohol extract of tunny fish meat (Germo Macropterus) was found to be consisted of two parts.

The one, dialyzable through cellophane tube against redistillated water, was considered to be potassium phosphate (dipotassium salt), and the other seemed to be a peptide which was hydrolysed into aspartic, glutamic acid, serine, glycine, alanine, lysine, and histidine. During the concentration of the alcohol extract, white crystal occurred, which was identified to be creatine.

64. Action of fumigants on the components of cereals. Part 2. Action of chloropicrin. Ihei IWATA and Yosito SAKURAI (Journ. Jap. Soc. Food and Nutrition 11, 175, 1958)

It was found that the solubilities of soybean flour for H_2O, 10% NaCl and N/10 NaOH, together, changed distinctly and the chlorine content of protein increased by the fumigation with chloropicrin. The changes of the solubilities by the fumigation with chloropicrin were greater than with tetrachloride.

The toxic action of the fumigated casein should be examined in details, for the external abnormalities were observed in rat fed on the casein fumigated with chloropicrin and methyl bromide.

Part 3. Action of halogen compounds. Ihei IWATA, Yuzi UCHIDA and Yosito SAKURAI (Journ. Jap. Soc. Food and Nutrition 11, 178, 1958)

The solubilities of soybean flour and the chlorine contents of casein fumigated with several halogen compounds were examined. When ethyl bromide was used as the fumigant, the changes of the solubilities and the chlorine contents of soybean flour and casein were as distinctive as chloropicrin or methyl bromide. When methylen chloride, ethyl chloride and ethylen chloride were used, their changes were as small as tetrachloride.

65. A volumetric method for the determination of a small amount of halogen in foods. Ihei IWATA (Japan Analyst 8, 149, 1959)

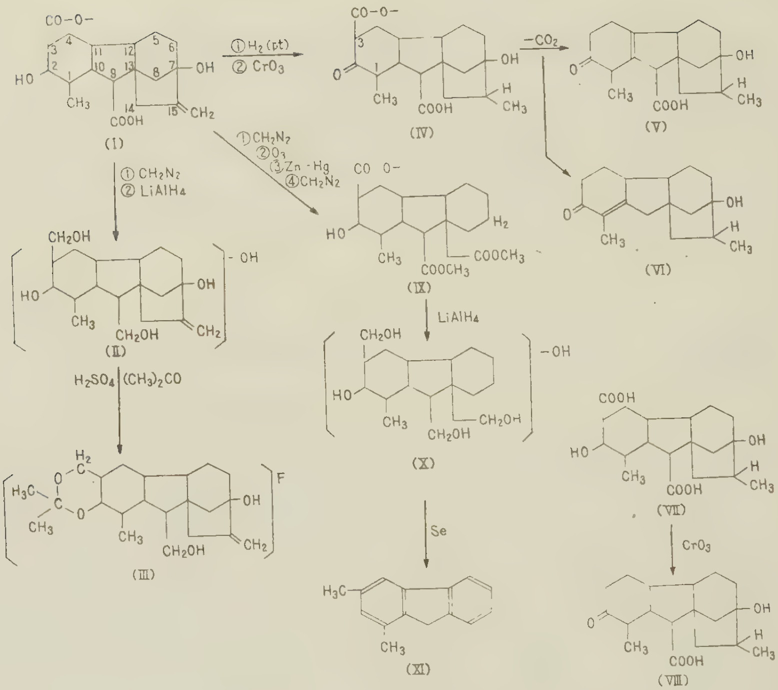
A high-frequency titrimeter with a cathode ray tube has been devised. The titration curve obtained showed the changes of the electric capacities of the measuring circuit, and the point of inflexion was observed on it. When sulfuric acid or hydrochloric acid was titrated with sodium hydroxide, the point of inflexion correspondeds to their equivalencies. When silver nitrate was titrated with 0.001 N ammonium thiocyanate, the error was within ± 0.03 ml. The determination of chlorine in sodium chloride was carried out with an accuracy of $\pm 2\,\gamma$ by the method of back titration. Furthermore, the chlorine content in foods could be determined without any interference from coexisting ions.

66. Studies on the change of chlorophyll by heating. Part 1. On decomposition conditions of chlorophyll. Osamu IGARASHI, Kenzo KOSUGE and Yosito SAKURAI (Journ. Agr. Chem. Soc. Japan 33, 281, 1959)

Between fresh tea leaves and tea products (especially, Sencha) there existed differences in their spectra of acetone extractive fractions. The spectrum of the former had maxima absorption at 430, 660 mμ and the latter at 410, 450, 665 mμ of wave length. As the same differences were found between raw and heated tea leaves, it was supposed that some decomposition of chlorophyll had occurred by heating. But, in spinach leaves, the change of spectrum was scarcely found by heating. It was found that the rate of decomposition of chlorophyll was influenced mainly by the pH of the leaves and by the heating temperature. When chlorophyll a separated and purified from spinach leaves was heated in pH 6.0 phosphate buffer, the same changes as heating tea leaves appeared, but in pH 7.0 the same changes as spinach. When the same treatment was carried out with tris-maleate buffers, the changes occurred not only at pH 6.0 but also at pH 7.0. While, chlorophyll b was scarcely changed by the same heating.

67. Biochemical studies on "Bakanae" fungus. Part 45. Chemical structure of Gibberellins. XII. Yasuo SETA, Nobutaka TAKAHASHI, Hiroshi KITAMURA and Yusuke SUMIKI (Bull. Agr. Chem. Soc. Japan 22, 429–431, 1958)

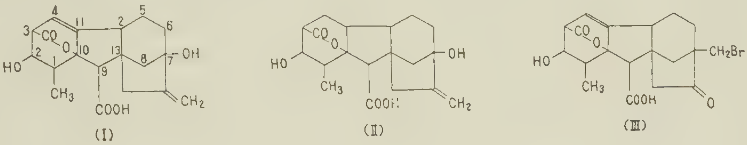
The lactone carbonyl of gibberellin A_1 has now been elucidated to be located at C-3 of the ring A, by the following experimental scheme.



Part 46. Chemical structure of gibberellins. XIII Nobutaka TAKAHASHI, Yasuo SETA, Hiroshi KITAMURA and Yusuke SUMIKI (Bull. Agr. Chem. Soc. Japan 22, 432–3, 1958)

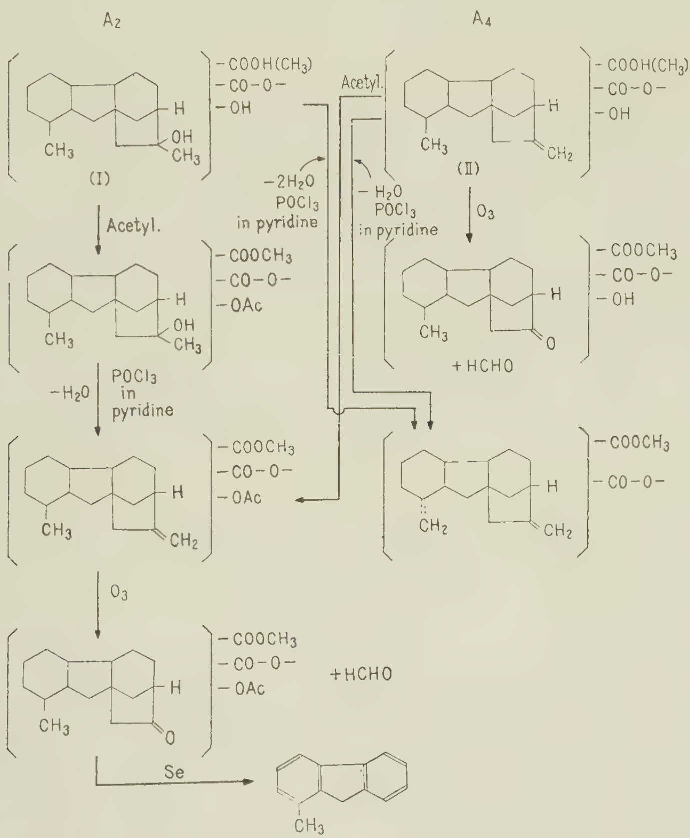
The attaching position of lactone alkyl oxygen and the final structure of gibberellin A_1 and A_3 were reported. When A_3 was treated with bromine in tetrahydrofuran at $-5°\sim0°$, monobromo-A_3 was obtained. Monobromo-A_3 gave the monobromo-A_1 in a 30% yield on catalytic reduction at neutral condition absorbing one mole of hydrogen. From these results, it is clear that the double bond in the ring A of A_3 remains unaffected in monobromo-A_3. Therefore, the monobromo A_3 was used to determine the position of the double bond in the ring A. Monobromo A_3 gave monoacetyl-monobromo-A_3 methyl ester by acetylation and methylation. When monoacetyl-monobromo-A_3 methyl ester was treated with ozone, a non-volatile acidic substance which showed to be monobasic by titration, was obtained. This result leads us to conclude that the double bond exists at the position of C-4$\sim$C-11. Because, if the double bond exists at the position of C-1$\sim$C-10, the oxidation product by ozone should not be a carboxylic acid but diketo compound. Moreover, when the ozonolysis product was boiled with 33% H_2SO_4, evolution of 0.5 mole of CO_2 was observed. The easiness of decarboxylation shows that the monocarboxylic acid is β oxocarboxylic acid. From these facts, we assigned the position of the double bond of monobromo-A_3 and A_3 to C-4$\sim$C-11. It has also been clarified that alkyl oxygen of lactone is allylic to the double bond, because of the easy occurrence of hydrogenolysis on the catalytic hydrogenation of A_3 and monobromo-A_3. As a result,

the structure of A_3 is formulated as (I) and those of A_1 and monobromo-A_3 as (II) and (III) respectively.



Part 47. Chemical structure of gibberellins. XIV Hiroshi KITAMURA, Nobutaka TAKAHASHI, Yasuo SETA and Yusuke SUMIKI (Bull. Agr. Chem. Soc. Japan, 22, 434-5, 1958)

The partial structures of gibberellin A_2 and A_4 were represented by formulae (I) and (II) respectively on the basis of the following experimental scheme.



68. Gamma irradiation of miso and soy sauce. Akira MATSUYAMA, Mitsuo NAMIKI,

and Yoshishige OKAZAWA (Journ. Fermentation Technology 36, 515–518, 1958)

Radiation effects on Miso and Soy sauce were studied, using the γ-irradiation apparatus (^{60}Co, 270 curies). Miso (or fermented Soy bean paste containing NaCl) generates gases on storage at relatively high room temperature, and this phenomenon, so called "Waki", is disadvantageous for commercial packaging. Irradiation is effective to prevent the gas liberation from Miso. The dose levels required for complete inhibition are 10^6r, depending on the variety of Miso containing NaCl at various concentrations (about 5-14%). The gas liberation from Miso, is considered to be caused mainly by yeast. Various types of microorganisms in Miso such as bacteria, yeast and molds were examined and found that they are incubated with the radiation treatment. Gases produced from Miso were found to be consisted mainly of carbon dioxide and alcohol, by the absorption test with 25% KOH an infrared analysis, resulting in no influence of radiation the composition of the gas phase. It is noteworthy that bacteria including lactic acid bacteria can hardly be recovered from the radiation damage, but yeasts recover easily. Such difference results in the modification of microflora in Miso. The post-treatment, heating at 60°C 15 minutes, enhances the radiation lethal effects on microorganisms in Miso. Color of Miso is slightly bleached by radiation, but the dark brawn coloring in storage at 30°C after irradiation proceeds faster than that of unirradiated control. The after-effect of radiation on the color of Miso more marked under aerobic conditions than under anaerobic conditions. The development of unfavorable off-flavors is detectable at a dose level of 10^5r. But the taste of Miso is hardly influenced by radiation, increasing slightly the salt taste only. Sterilization effect of radiation on Soy sauce is not so remarkable, in spite of its high NaCl content (about 18%). Particiular off-flavors from Soy sauce developed above a dose level of 10^5r. However, the irradiation treatment with the low doses shows no detectable undesirable off-flavors, i.e. irradiation by $7\text{-}20 \times 10^3$r seems to improve the quality of Soy sauce in taste and flavor.

From the results above, the important problems to be solved in view of practical application of radiation are the following two. One is the enhancement of the total lethal effect on microorganisms in foods and another is the inhibition of the undesirable side reactions such as the off-flavor development, color change, etc.

III. Agricultural Economics

Abstract Nos. 69

69. **Rice-farming and Irrigation in Japan.** Natsuki KANAZAWA (Summary of report Farm Management Development Center of F.A.O. 1957)

In Japan, rice farming is the nucleus of her agriculture. It goes without saying that the climate of high temperature and high humidity peculiar to this country place rice growing with under favourable conditions. From ancient times, Japanese farmers have been devoted chiefly to rice farming. In the age of our old feudal lords, the land tax in kind used to be represented by rice and, until the postwar agricultural land reform as well as in the prewar times, the land-rent was generally paid in the form of rice. In the present-day Japan, a multitude of crops, including many kinds of truck

crops, are cultivated. However, rice cultivation is more generalized than any of such crops. The number of rice farmers, whether they cultivate it either for sales for commodity or for home-consumption, constitute some 90% of the total farmers in this country. The rice farming in Japan, though partially carried out in upland fields, is usually done on paddy fields. The area of her paddy fields totals some 3 million hectares, approximately 55% of the total cultivated land.

No one could doubt that irrigation is playing a great role technically and socially in her rice farming or paddy field farming. A true recognition of the status of such irrigation would be a key to the better understanding of the patterns of Oriental society. The Oriental agriculture, as significantly described in such an expression a 'No farming cannot exist apart from water', has been characterized by the fact that farmers have been devoted to overcome the problem of how to control, obtain, distribute and use water. This is also true of the Japanese agriculture, and it is safe to say that Japan's agriculture has been always as much coexistent with water as European agriculture with domestic animals. Accordingly, I would like, a this juncture, to speak briefly of the status of Japan's irrigation practice, farm management, and water utilization.

(1) Water and Rice Production.

The study of the rice farming hitherto conducted in Japan shows that the water requirements in the rice farming period vary from 900,000 to 1,400,000 l. per "tan" (approximately ¼ acre). However it is reported that water requirements of paddy rice (quantity of water need to make one gram of dry weight) are less than those of other crops. For instance, the water requirements of paddy rice are nearly 300 grams, being below those of potatoes, etc. as well as barley and wheat. To put it in another way, this means that the quantity of water directly needed for rice as a kind of plant and the water needed for rice farming assume a considerable difference of significance between the two. In short, rice could attain its soundest growth in such a state as water is filled on paddy fields and consequently irrigation would be the main vehicle to place paddy rice under the most favorable environment of growth. Thus the major purpose of irrigation is to assume the role of keeping the water warm, supplementing nutrients, controlling weeds, etc., in concert with land.

The optimum temperature for rice growth is 32°C. In Japan, however, where her temperature is lower than the said optimum in more than half the period of rice farming, the filling with water on paddy fields itself is effective in lifting water and under ground temperatures to higher degrees. Generally, there is a difference of 8°C between water and underground temperatures in an early stage of rice farming. And irrigated water itself occasionally contains nutrients in a volcanic country like Japan, including abundant potash and silicic acid. For instance, in paddy fields located in the Shirakawa river basin in Kyush (southwestern part of Japan) is supplied potash of 30 kg. per acre by means of irrigated water; and a computation indicates that silicic acid of 60-240 kg. per acre, averaging some 90 kg., is supplied in the rice farming period. Non-use of phosphatic or potash fertilizer often does not result in decreases in yields even after the lapse of several or sometimes more than ten years due to the circumstances mentioned above. One could easily imagine how effective the filling with water on paddy fields is in controlling weeds in comparison with upland field cropping. The direct seedling culture of rice is faced with a difficult problem of weed controls. In this method it is said to be required weed controlling manpower twice as

many as that required for the filling with water on paddy fields.

The percentages of weeding labor required for paddy and upland rice fields against the total rice farming labor are 17.5 and 46.7% respectively.

Planned distribution of water

	Degree of water requirement	In the case of a little water	In the case of least water
1. Stage when transplanted seedlings take roots	Most necessary	Ordinary watered	Shallow or damp
2. Early stage of tillering	Necessary	Damp	Suspended
3. Middle stage of tillering	Necessary	Do.	Do.
4. Late stage of tillering	Necessary or least necessary	Suspended	Do.
5. Initiating stage of young spikes	Most necessary	Saverally irrigated	1–2 times irrigated
6. Stage of reductive division	Most necessary	Severally or 1–2 times irrigated	Do.
7. Heading and flowering stage	Necessary	1–2 times	Damp or suspended
8. Green ripening stage	Necessary or Less necessary	Damp	Suspended
9. Yellow ripening stage	Less necessary	Suspended	Do.
10. Fully ripening stage	Least necessary	Do.	Do.

Ther rice farming will be fulfilled most efficiently only at a time when irrigation can play the said roles to the full extent. In short, it would be possible to the extent on the basis of rationaly planned distribution of water. The key to determination of rice production is how to exercise planned distribution of water for an adjustment of the depth of water on rice fields or that of the irrigation or drainage timing. Any of successful rice farmers in Japan who could have hitherto harvested high-yielding rice (700-900 kg per ¼ acre) has a knack at a planned distribution of water. However, a prerequisite to this consolidation of paddy field with perfect equipment of water ways is necessary. In Japan, however, a considerable number of paddy fields have undergone a partial improvement such as drainage or top soiling, etc. and badly subdivided into too small plots in regard to any of farms. In addition, the adjustment of partitions of paddy fields also is comparatively scarce and very few are perfectly equipped with improved water ways or agricultural roads. One of the important problems which

Japanese agriculture has to solve is to develop cultivated land into better conditions.
(2) Rice Farming Patterns and Irrigation in Japan.

According to the "Survey of Irrigation Status by Watershed" made by the Ministry of Agriculture and Forestry Japan's irrigated land by water resources can be divided into the following composition: some 2,170,000 "cho" (1 cho approximately equivalent to 1hectare) of river irrigation, 68.3% of the total area concerned; some 570,000 "cho" of reservoir irrigation, 18.3%; some 50,000 "cho" of lake and marsh irrigation, 1.6%; some 170,000 "cho" of underground water irrigation using wells, 5.3%; 210,000 "cho" of other irrigations, 6.5%.

Excepting paddy fields depending upon lakes, marshes, spring water, and rainwater for their irrigation, Japan's rice farming relies chiefly upon two kinds of water resources for irrigation, i.e., rivers and reservoirs. Needless to say, the distinction between rivers and reservoirs only represents those between the two patterns of water use. Therefore, the fact that most of Japanese paddy fields depends chiefly upon the said two water resources cannot be immediate criteria to study her rice farming patterns.

Nevertheless, I do believe that the distinction between such irrigation patterns, as described below, would be an important key to better understanding of her rice farming patterns. Of course a mere expression of river irrigation means all kinds of river irrigation such as natural and artificial rivers, big and small rivers, streams irrigated into paddy fields in mountainous localities and so on. And this is true with reservoirs which are considered to be different in nature according to structural divergence resulting from varied topographical conditions and to their new or old history. Therefore in this regard, any sweeping discussion would be quite unreasonable even if such situation is taken into account, however, the difference between these two irrigation patterns seems to show the features peculiar in sharp contrast with the rice farming management in Japan.

In a word, reservoir irrigation is mostly prevalent in the southwestern part of Japan and, roughly speaking, river irrigation in the northeastern part. The irrigation history of this country immediately represents the history of the development of our civil engineering. In such meaning, the history of our reservoir irrigation system is comparatively old. Especally in the mountains we occasionally can see old reservoirs built more than 1,000 years ago by taking advantage of hollow parts of the mountains. Besides, in plains are seen old reservoirs with shallow bottoms, of which the water is usually supplied from neighboring small rivers. Some of the oldest river irrigation type, among the used still those utilizng small rivers or mountain streams are intercepted by heaping timbers or stones for that purpose. Ever since the Tokugawa Shogunate regime in Japan, i.g., during the period from 17 century through mid 19 century, agricultural reservoirs had been constructed by warlords who used to accomplish their works to admit the water of small rivers into plateaus by using their peoples labour. Until that time, little progress was made in using the water of big rivers for irrigation purpose in plains due to the imperfectness of water control works. The Shinano or Tone River, most typical among the biggest rivers in Japan, had never been used for irrigation purpose before our water control works were developed in the Meiji era. In the irrigation system using big rivers for irrigation purpose, irrigation structures are modernized with big reinforced concret dams, headeworks, operating watergates and other structures. Especially watergates equipped with unified inlets

ar considered most modernized.

In Japan two types of irrigation system—long used and very antiquated ones and awfully modernized ones—are co-existing with each other. Generally speaking, however, many of the irrigation systems using the water of big rivers are modernized. Of course there are many modernized reservoirs, especially big ones most typical. One of the features distinguishing the reservoir irrigation system from river irrigation system is the diffeence of admitting quantity of water.

As shown in the table of next page, the river irrigation system has more abundant natural flux of water than the reservoir system. On the contrary, it is self evident that underground water resources or "others with no particular water resources" occasionally cause water shortage. However the figures shown in table only account instead of natural flux of water per unit area for surplus or shortage of water practically used by farmers for their farm management. Even if the natural flux of water is shortcoming, farmers may sometimes obtain sufficient water artificially through other auxiliary means. In other times, more intensive farm management may result in water shortage.

Irrigation by Water Supply Resources	Most abundantly irrigated area	Appropriately irrigated area	Water area shortage
River	20.2%	56.0%	23.8%
Reservoir	12.8%	52.1%	35.1%
Lakes and marshes	23.2%	58.6%	18.2%
Underground water	15.05%	49.6%	35.35%

In short, abundance or shortage of water is caused not by natural flux of water but by farm management patterns. Generally speaking the more intensive farm management becomes, the more water is needed. In the southwestern part of Japan where the rice plant management depends chiefly upon reservoir irrigation system, the shortage of natural flux of water was supplemented by using pumps or wells. In the southwestern part of Japan, rice farming requires more labour input for irrigation purpose than in the river irrigated area of the notheasten pat. For instance, in the northeastern part of Japan including Niigata, Ishikawa or Yamagata prefecture labour input needed for irrigation or drainage in river irrigated areas accounts for only 3-4% of the total labour input for rice farming, while in Hyogo or Kagawa prefecture for 9-14%. In the prewar time, such typically reservoir irrigated areas were called most developed in rice farming in Japan. The disadvantage of natural conditions compelled farmers in those regions to adopt supplementary means, resulting in stabilization of rice-plant management. On the contrary, in the big river irrigated areas, such abundant natural flux of water had never been utilized to the full extent due to the unskillness of our civil engineering sufficing for irrigation purpose. Therefore it may be said until recent years, river irrigation was in a state closer to nature than reservio irrigation. In fact shortage of water occurred very often in river irrigation areas. The features of a reservoir is its ability to supply a rather small but constant quantity

of wate. The reservoir, in which water is to be stored in the non-irrigation period, is less subjected to annual variations in temperature than rivers. The rice farming, if water could be supplemented with artificial auxiliary means, would be put on a more stable basis. Nevertheless, the river irrigation system, in fact, results in unstable supply of water year by year, occasionally bringing a great diffeernce of water supply between the abundant and shortcoming period. In the northeastern part of Japan, the rice farming, in spite of its great potentiality of development from the viewpoint of natural supply of water, has long been far behind the southwestern district up to the latest years. For the construction of reservoirs can be easily feasible in terms of civil engineering and carried out on the smaller scale basis than the river irrigation system. In othr word, the latter would be feasible only so far as a large sum of investment in civil engineering can be accepted as a first prerequisite. The Japan's rice farming s very often said to be like horticulture. This criticism holds all the more true with the rice farming developed in reservoir irrigated areas. After the war-end, our rice farming has made a remarkable development in the river irrigated areas located in the northwestern part of Japan. With gradual completion of land improvement and irrigation system, however, those areas where relatively large-scale farming has been traditional, they are steadily increasing their productivity hitherto not realized to the full extent.

For better understanding of the status of the Japan's rice farming, one should keep in mind that reservoir irrigated areas situated in the southwestern part of Japan where rice farming has been formerly on the small-scale but stabilized basis in the lower stage of our civil engineering and that the river irrigated areas in the northeastern part where rice farming has been on the traditionally larger scale basis, have been forced to remain at the lower level of productivity while submitting to natural agencies with all their high productive potentiality, but recently they are steadily increasing their productivity as a result of the technical progress and land investment in this country.

(3) Irrgation Customs and Organization.

The manner in which farmers in practice use water for their farm management is mostly pursuant to old customs. The customs involved in irrigation water supply are so complicated in Japan that full analysis of their status is not easy. With the recent progress of analysis in this field, however, the status of such irrigation water customs is being considerably discolsed. At present, those customs are legally effective as common law.

The reason why our irrigation practcie has been hitherto left to customs is due to the great intricacy and unmanageability of such problem in addition to the obscurity of its status. Above all, in the reservoir irrigated areas situated in the southwestern part of Japan, the irrigation water customs are provided for in their detailed parts due to the considerable narrowness of such areas, constant quantity of water supply, relative deficiency in water supply and so on, being awfully complicated and strongly restrictive as well. In such situation, rice farming accept such customs as given conditions supporting it and within the limits of such acceptance, adapt itself to them. Generally speaking, most of our irrigation customs provide for the quantity and period of time of irrigation water supply for paddy rice transplanting. As a result, the transplanting season of paddy rice is totally limited and individual farmers make efforts to adapt their other phases of operations to such limitations. Roughly speaking,

the technology of our rice cultivation has made a development to adopt itself to those condiions limiting such rice transplanting. It is safe to say that the irrigation customs have been so much powerful in social restraints. One of the reasons for that is that the transplanting operation is most important among diverse operations required for paddy rice production, and that, the significance of land owners is role in implementations of those customs in the prewar Japan should be taken into account. The management of our irrigation customs has been left to some of the land owners of the upper classes under the guise of the interest of the entire farmers concerned.

Before the war, the landowners had a great influence, constituting the nucleus of all the fields of their community life, production and social activities. Accordingly the irrigation system put into practice pursuant to such customs, in effect, might bring about considerably different interests among individual farmers. It was only natural that land owners could be in a more favorable position under such irrigation customs. By small tenant farmers, water use of individuals was incompatible with that of land owners.

Such situation was all the more serious with the reservoir irrigation system in which customs have detailed regulations designed for repetition of the similar situation. The river irrigation system also is involved in many customs. In this case, however, it is rather difficult to have detailed rules due to extensiveness of rrigated areas and, for that reason, fundamental principlestare only provided for. Besides, imperfectness of irrigation facilities is apt to cause an uncertainty of water supply as a result of annual variations in rainfalls and to make those customs themselves difficult to be effective. Our land reform weakened the traditioned influence of landowners and the effectuation of the Land Improvement Law of 1952 changed the Irrigation Associations which had ever been bodies for landowners in the past to those for cultivators. This fact will be able to give us a great strength to transform our traditional irrigation customs. In fact, however, transformation of irrigation customs is only taking place in Japan with a very slow step. Because farmers have been engaged in their farm management by adapting to the unreasonableness of such irrigation customs, and rationalized them through self-adapting means of their own. In addition, complicated procedures of distributing water in the community concerned result in specialization of the persons in charge. In such situation it is difficult to change the present persons in charge for someone else.

To start the farm management in the right directions, any irrigation system should not be made given condition but freely available to every individual. Even now, farmers adhere to old customs as they are by developing their adapting means. Under such circumstances, the development of farm management would inevitably come to the limits.

(4) Future Problems.

In recent years, however, we have some examples in which our irrigation customs are in the process of steady collapse. The post-war's remarkable increase in agricultural productivity in Japan is about to break up such old irrigation customs as given conditions. For instance, we can enumerate an early culture of paddy rice, conversion from paddy to upland fields, etc. However, any management for such purpose would not be feasible without changing the old rules of our irrigation system. Besides the direct seedling culture of rice-plant or dissemination of weed killer 2.4 D, etc. could be most effective under such circumstances as possible irrigation or drainage to be carried out

on the best planned basis. Be that as it may, such technology as early or direct seeding culture of rice plant, and of which proved not to be feasible within the limits of our old customs irrigation and required their changes, in fact could not make any development in regions bound by strong customs. In the past, auxiliary technique could make a progress in proportional terms in the regions of strong customs and additionally realized higher yield of rice. Such being the case, the said technique practically was introduced at the start only in lower yield regions. In this connection, farm mechanization requires collectivization of paddy fields with increased acreage of each tract. For that purpose, consolidation or readjustment of partitions of paddy fields should be made with inevitable alternation or improvement of irrigation channels. And if so, our old irrigation customs will be seriously threatened. In the past, Japanese rice farming has surely made a long stride in technique at handicraft requiring small amount of capital in adapting itself to complicated irrigation customs and assisting such adaptation. In the future, however, the urgent necessity would be to place paddy fields under conditions sufficiently favorable for cultivated land conditions. For that purpose, larger amount of investment would be needed for land improvement or irrigation system. Thus our agricultural production will be capable of further growth, the Japanese Government has launched to increase investment in that field.

However, the strength of increasing agricultural production, in a true sense of the word, lies in farmers themselves. Any Government policy such as agricultural land reform or civil engineering would be nothing but incentive to encourage farmers. None the less, such policy should play a role as, as it were, lubricating oil in helping farmers to develop their farm management. Because farmers don't always respond to large-scale projects initiated by the Government and be healed of their discontent with the existing irrigation arrangement. Furthermore, in order that the State's projects can be truly contributive towards farm production, long-term efforts on the part of farmers for their better adaptability would be a prerequisite. The State occasionally is apt to lay one-sided plans for increased agricultural production and wishes to put them into practice. However, such practice does not necessarily please farmers at all times because they are the parties responsible for farm. It is of great importance that the State's policy should always look in the face an internal changes of farm management and be embodied in light of the timing of the prospective maturity of such change. Inasmuch as any improved agricultural irrigation would be difficult by means of individual farmer's endeavors, it is all the more desirable to keep this point in mind.

IV. Agricultural Engineering

Abstract Nos. 70—84

70. Mechanism of soil moisture extraction from a pressure-membrane apparatus. Hitosh FUKUDA (Highway Research Board, U.S.A. Special Rep. 40, 73–77, 1958)

In this paper a mechanism by which soil moisture is extracted in the case of the pressure-membrane apparatus developed by Richards was considered theoretical. And the effects of air humidity and room temperature outside the apparatus on soil moisture

in equilibrium with various pressures inside the apparatus were experimentally proved to be of little importance.

71. Intake test for special apparatus. Shozo NOGUCHI (Study on the water temperature 2 [5], 1958)

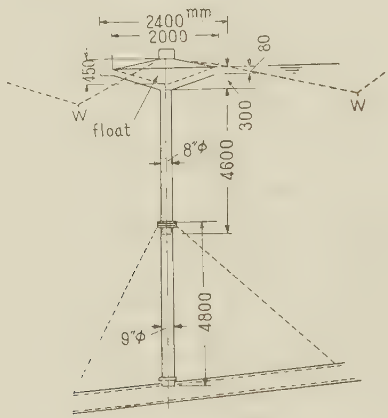
The experimental apparatus is invented by Mr. K. ITO as shown in Fig. 1 to take only surface water in reservoir for irrigation.

By working the apparatus, about 200 l/sec of water is able to take.

To obtain basic data for the flow of water in the apparatus, experiments were made concerning a few items.

The obtained results are as follows.

1. Working this apparatus, only surface water of reservoir (from water surface till about 40 cm depth) is taken out very smoothly, and cool water is not taken.
2. By this apparatus, the temperature difference between surface water of reservoir and intake water is 0.6°C or lower than this, while by the old type apparatus (orifice-type), the temperature difference is about 1.5∼2.0°C.
3. The experimental apparatus is very small type, but setting large type, it will be able to take more larger amount of water.
4. When working this apparatus, the velocity of inflow water must be smaller than the critical velocity.



72. Some consideration of the hulling function of Centrifugal Rice huller and the hulled property of paddy. Fusanobu SHOJI (Journ. Agr. Machin. Soc. Jap. 20 [2], 83-87, 1958)

In this paper the author practiced measurements on the hulling function of Centrifugal Rice huller, giving one paddy at a time under various revolutions of the impeller (diameter is 230 mm). The results obtained are summarized as follows.

1. The lower limit of revolution of the impeller is about 500∼1000 rpm for hulling action, and the upper limit about 4000 rpm.
2. The relation between revolution of impeller and hulling rate of paddy is shown by S-figured curve.
3. The characteristics curves of the hulling property of paddy to given revolution

of impeller are similar to the normal curve of errors.
4. Broken rice is assumed to be chiefly occurred by the second impact-process.

73. **A consideration on the three wheel type of small riding tractor.** Fusanobu SHOJI and Fumihiko SANO (Journ. Agr. Machin. Soc. Jap. 20, [3], 113-118, 1958)

As a partial study on the small riding tractor, authors investigated various kinds of the three wheel tractor at home and abroad. On inquiry, we understood that there are 8 kinds of threewheel type on classification as shown in following table and clarified their characteristics, scrutinizing their capacities of stability, steering and running, and some problems for all purposes.

No.	front wheel	rear front	steering wheel	drive wheel
1	Single wheel	two wheels	front and rear wheel	rear wheels
2	dual 〃	〃		
3	double 〃	〃	front wheel	front wheel
4	single 〃	〃		
5	two wheels	single wheel	front wheels	rear wheel
6	〃	〃	rear wheel	
7	〃	〃	front & rear wheel	front wheels
8	〃	〃	front wheels	

74. **Studies on the distribution of threshed materials under the concave sieve of threshing machine. Part 3.** Fusanobu SOJI, Hiroshi MORISHIMA (Journ. Agr. Machin. Soc. Jap. 20 [4] 167-170, 1959)

The authors studied the distribution of threshed materials under the concave sieve of a threshing machine which applied to over-feed system. The distributions of threshed materials were almost the same as those of under-feed system, showing W figure type at one man operating and U figure at two men operation.

However, they pointed out some differences between these two feeding system. Authors clarifid the main factor which characterized the over-feed system derived from the formation of higher effect depended on the relative position among concave sieve, cover casing and action point of threshing cylinder, illustrating the free distribution of threshed materials of a pedal threshing machine, as the original model of this system.

In addition to that, they maintained to explain the custom of applying the over-feed system to the treatment of comparatively big bundle, was regarded to the effect of the size of bundle on the distribution of grain.

75. **Study on the bearing power of the peat soil.** Fusanobu SHOJI and others (Journ. Agr. Eng. Jap. 26 [6], 330-337, 1959)

The authors practiced load test and penetration test in order to estimate the bearing power of peat soils, and moreover, conducted physical and consolidation test on the soil. Thus, we investigated the relation between physical characteristics and bear-

ing power of soil.

In case of constant sinking, it seems that a certain constant relation exists between the intensity of load and circumferential length of a loading plate, but practically a considerable deviation is seen between them. Moreover, their relation is always shown by a curvilinear one. In this case between the ratio of circumferential length to the area of the loading plate, or the circumference-area ratio and the intensity of load there exists the following relation:

$$p = mx + n$$

where p=intensity of load, x=circumference-area ratio (length of circumference/area), m, n=constants.

And the authors practiced the penetration test as a rapid and simple method in order to obtain data for estimating the bearing power of soil. The penetration resistance corresponds to the generating layer of the peat soil, quite well. And in the order of high-, medium- and low-humused peats, it was regarded that the degree of decrease of penetrating resistance was getting larger.

As the result of the physical and consolidation tests, it was learned that peat is rich in the character of consolidation, and this property is more remarkable in highly humus peat soil.

In short, the peat soil are easily subjected to consolidation, and as the bearing power of them is very small, the introduction of heavy machinery, or construction of structures should deliberately be put into consideration.

76. On the statical sinking of heavy tractors on the peat ground. Fusanobu SHOJI and others (Journ. Agr. Eng. Jap. 27 [1], 17-20, 1959)

When we intend to work heavy wagons such as tractors or bulldozers on the peat ground, we are faced with the problem of the bearing power of the ground. So, at the first stage of this research, the authors investigated the bearing power of peat, making use of loading plates of various sizes and shapes, and examined the relation among the load, the required time and the sinking degree.

On the basis of the conclusion of the research above, this paper explains the results of the experiments on the sinking amount of low-humused and medium-humused peat soils, which are unstripped and 20 cm stripped of the surface, under the real static wagons.

Five bulldozers or tractors with track-shoes of different shapes and giving various pressures on the ground surface were used in the experiments. The results are summarized as follows:

As in the case of the loading plate, the intensity of the loading weight for the constant sinking is influenced by the circumferential length and the area of the ground-touching part of the machine. It is in direct proportion to the ratio of the circumferential length to the area, and when other conditions are the same, it is dominated by the shape of the ground-touching part of the track-shoes.

77. Studies on the mole-drain. Part 2. Fusanobu SHOJI and others (Journ. Agr. Eng. Soc. Jap. 36 [4], 181-196, 1858)

As the method and efficiency of operation concerning the execution of mole drain are influenced by the soil condition of paddy fields, the authors practised an experiment in field on the above problem.

The tractive resistance of mole drainer under the paddy fields (depth of drains; 60 cm) averages 350 to 400 kg for peat soil, 500 to 700 kg for clay and clay loam, and 800 to 1,000 kg for loam and sandy loam; in case moisture content is much even if soil type is similar, the tractive resistance becomes smaller.

If the soil condition of paddy fields is expressed by soil hardness p (measured by means of a cone-penetrometer), the changing figure of soil hardness between the soil depth h(0-90 cm), or the phase of soil hardness has in general 4 kinds of different types from the view point of model type. The above noticeable types are shown as (1) one mountain type, (2) two mountain type, (3) terrace type and (4) mountain side type.

Approximately the following relation exists between the hardness of paddy fields an tractive resistance Q (kg) of mole drain:

$$Q = pm(A + \alpha B)$$

where pm=average soil hardness (kg/cm^2), A=cross-sectional area of drain (cm^2), B=cross-sectional area of supporting edge plate (cm^2) and α=coef. of changing by structure of drainer, depth of drain and soil condition (in this case the value of 3.0 may be practically adopted).

By this formula they can estimate the required tractive resistance only in measuring soil hardness, before the execution of mole-drains is started.

78. **Transistorised tele-torquemeter.** Yoshichiro YASUDA and others (Ann. Rep. Eng. Res. Inst. Fac. Eng., Univ. Tokyo, 18 [1], 1–6, 1959)

Previous experiments show many defects; such as extra low S/N ratio and so many improvements have been made as described below.

The mobile unit's carbon ring was replaced by silver ring torque pick-up.

Complete redesigning in the electronic system was also made including FM transmitter, oscillator, audio amplifier and receiver, permitting to work on threshold level of 50 μV.

The transmitting antenna was also replaced by ferrite cored bar antenna system.

Experiments on the whole system is expected to be carried out as soon as possible.

79. **Studies on oceanic salts. Part 4. A new basic salt containing Magnesium Hydroxide (1) The quaternary system K_2SO_2–$MgSO_4$–$Mg(OH)_2$–H_2O at 100°.** Jiro SUGI and others (Sci. Papers of Central Res: Inst. Japan Manopoly Coorporation 101, 114–122, 1959)

Two new basic salts containing magnesium hydroxide were found and these were named "NS salt A" and "NS salt B". The chemical formulae of these salts were decided to be as follows.

NS salt A $Na_2SO_4 \cdot 2MgSO_4 \cdot Mg(OH)_2 \cdot 4H_2O$

NS salt B $K_2SO_4 \cdot 2MgSO_4 \cdot Mg(OH)_2 \cdot 2H_2O$

If "Ns salt B" were to be separated from bittern, it would be possible to separate potassium dissolved in bittern as sulfate directly. Therefore, to extract NS salt B from bittern efficiently the 100° isotherm of the four component system K_2SO_4-$MgSO_4$-$Mg(OH)_2$-O_2H and associated systems K_2SO_4-$Mg(OH)_2$-H_2O and $MgSO_4$-$Mg(OH)_2$-H_2O were examined. Though the isotherm K_2SO_4-$Mg(OH)_2$-H_2O exhibited nothing new, isotherm $MgSO_4$-$Mg(OH)_2$-H_2O exhibited basic magnesium hydroxide $MgSO_4 \cdot 5Mg(OH)_2 \cdot 3H_2O$ as the solid phase. The solid phases of quaternary isotherm were NS salt B, langbeinite, basic magnesium sulfate, kieserite and potassium sulfate.

80. Use of ion exchange membrane in the electrolytic concentration of sea water. Part 2. Jiro SUGI and Others (Sci. Papers of Central Res: Inst. Japan Monopoly Coorporation **101**, 142–144, 1959)

The constituents of electrodialysate of sea water obtained by the use of ion exchange membranes were examined. Na content was calculated from ion balance after Ca^{++}, Mg^{++}, K^{+}, Cl^{--} and SO_4^{--} were analysed. NaCl ratio for total salts in concentrated dialysate was 77-76%. K^{+} in concentrated dialysate was richer than that in sea water on brine of RYUKASHIKI (Flowing down salt field) while the SO_4 ratio for total ions was less.

81. Studies on the protection of salt making apparatus from corrosion. Part 2. Use of corrosion inhibitors, in descaling of salt making apparatus with acids. Jiro SUGI and Others (Sci. Papers of Central Res: Inst. Japan Monopoly Coorporation **101**, 173–185, 1959)

We added various inhibitors to the acid used in cleaning such as picking off metal surfaces and dissolving off scales. Almost all inhibitors available on market were effective for iron and steel, but only a few were found effective for copper and coppery alloy.

We studied anti-corrosive effects of various inhibitors and discussed the inhibiting mechanism. Moreover, we investigated the application of particular surface active agents of the cationic type, which was ascertained to be remarkably effective as a corrosion inhibitor.

Anti-corrosion effects were determined using 1.05 N hydrochloric acid at 25°C, as a comparative test and 1.05 N HCl at boiling point as a practical example. The characteristic electric resistance was also measured by a simple method.

It was found that Stearamidomethyl pyridium chloride was most effective for iron and steel, and with compounds of Thiourea its effect for copper and coppery alloys increased remarkably. This inhibitor was far superior to those hithertofore used.

82. Studies on alkali and alkali earth metals selective exchange resins. Part 2. On the synthesis of Potassium Exchanger Jiro SUGI and Others (Sci. Papers of Central Res: Inst. Japan Monopoly Coorporation **101**, 145–148, 1959)

We synthesized a Potassium Selective Exchanger (SPAR) which is characterized by the possession of nitro and hydroxy radicals.

It absorbed potassium, about 90% of the total absorbed ions, from a 1:1 molar solution, containing Sodium and Potassium ions.

83. Studies on characteristics of salt, sea-water, brine and bittern. Part 3. Elevation of boiling point in brine and bittern. Jiro SUGI and Others (Sci. Papers Central Res: Inst. Japan Monopoly Coorporation **101**, 90–95, 1959)

Brine and brine treated with $CaCl_2$ for removing SO_4 were evaporated to various concentrations and elevation of boiling point of these solutions were measured at atmospheric pressure.

In solutions of the same Be° the boiling point elevation of the treated solutions were larger than that of concentrated brine, but the NaCl content was lower.

84. Salt for marine food preservation. Part 1. On salt containing antioxidants. Jiro SUGI and Others (Sci. Papers of Central Res: Inst. Japan Monopoly Coorporation **101**, 72–

79, 1959)

A method of dispersing antioxidant, BHA and BHT homogeneously into common salt was examined.

We suspended the antioxidant in saturated salt solution from the aid of non-ionic surface-active agent Twee-20. In this suspension salt was dipped and separated from the slurry by a centrifuge.

As BHT, auto-oxidizes, the salt containing BHT becomes remarkably yellow. The same yellowish color is developed on the surface of the fish salted with this salt.

We could prevent the coloration of the salt by adding a small amount of Citric acid, Calgon, or E.D.T.A.

Part 2. Salting of salmon and whale meat. Jiro SUGI and others (Sci. Pepers of Central Res: Inst. Japan Monopoly Coorporation, 101, 80–88, 1959)

1. Coarse Grain Salt (Koshitsu Soryushi), Imported Salt (Appropriately crushed for salting salmon) and domestic salt (Produced by Sakito-Seien) were examined.

SHE 107 B (A sort of coarse grain salt) was satisfactory. No appreciable difference was found among others.

2. Prevention of deterioration due to oxidation of oil in salted pink salmon, by addition of antioxidant BHA.

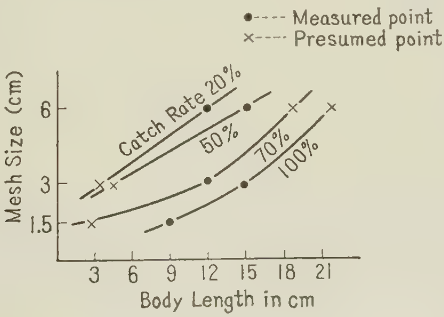
After storage for 4 to 6 months, salmon was examined organoleptically and chemically. Results showed that salt treated with BHA was effective against deterioration as compaired wih untreated ones.

V. Fisheries

Abstract Nas. 85—120

85. **Fishing effects on a model lift nets in regard to relation between mesh size and fish length.** Yoshio HIYAMA, Takaya KUSAKA and Yoshiaki YAMASHITA (Bull. Jap. Soc. Sci. Fish. 24, 466–468, 1958)

As a fundamental experiment concerning effect of lift net, the authors studied the relation between mesh sizes and fish lengths of catches in model lift nets.



The set of the observation was a tank of 80, 92 and 88 cm. (depth), as a material number 197 individuals were taken into consideration on the population to be approached number 197 individuals were taken into consideration on the population tube approached under the natural condition. Net materials were used of mesh size 6, 3 and 1.5 cm. and the lifting speeds of the net were selected 3 grades at 5, 10 and 20 seconds per 88 cm. The results were obtained from the number of the fish lifted and the catching rate. The figure was shown as an effect of what is an indicator of catching efficiency against a certain mesh size and fish length.

86. Fish gathering effects and submarine illumination of the incandescent and fluorescent mercury-vapor lamps. Takaya KUSAKA (Bull. Jap. Soc. Sci. Fish. 25, 17–22, 1959)

On the Tachibana Bay of Nagasaki Prefecture purse seine anchovy fisheries, using light, is carried on. The author investigated the fish gathering effects on lamps. One incandescent lamp of 2 KW (1 KW, 2 globes, D.C. 100-120 V) was generally used and another fluorescent mercury-vapor lamp of 2 KW (1 KW, 2 globes 100 V, A.C. 60 cycles) was specially prepared for this experiment with a 6 KW A.C. dynamo on a boat. The author compared the submarine illumination under each lamp and also the fish gathering effects on each lamp.

The fish gathering effect on the mercury vapor lamp was better than the incandescent lamp. This was especially true during the early period after lighting, when fishes of comparatively medium length gathered (scombrid and so on). In this case the fishery was mainly for anchovy. The fish school that gathered under the mercury-vapor lamp were swimming actively. However, during the later stages on the illumination of the mercury-vapor lamp it was found necessary either to weaken the illumi-

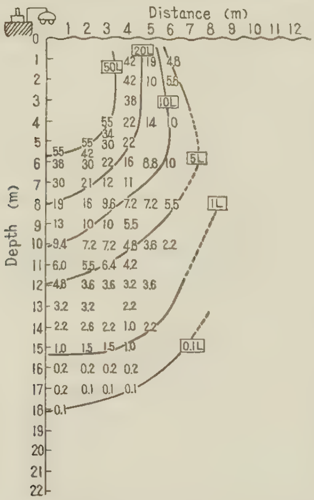


Fig. 1. Submarine lightness of 2KW Incandescent Lamp (Lux)
1 KW. 2 globes 120 V, D. C., off the coast of Obama, Aug. 20th, 1958, transparency 9 M. under the lamp.

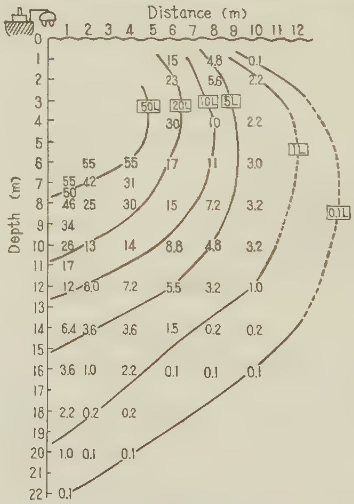


Fig. 2. Submarine lightness of 2KW fluorescent mercury-vapor Lamp(Lux)
1 KW. 2 globes 100 V, A.C. 60 cycles, off the coast of Obama, Sep. 2nd, 1958, transparency 9 M. under the lamp.

nation to change the lamp to the incandescent variety or to find some means by which the illumination of the mercury-vapor lamp could be maintained. The author supposes that the effective illumination for the gathering of anchovy is below 0.1 *Lux*. The illumination of the mercury lamp is about 30% more than that of the incandescent lamp and this is proportional to one dimension of length in water. (Figs. 1 & 2)

87. **A measure of role of utilization of plankton-feeders by carnivorous fishes in a certain area.** Fujio YASUDA and Yoshio HIYAMA (Rec. Oceanogr. works, Jap. 5, [1], 85–90, 1959)

In discussions on the productivity of plankton-feeders, if we want to know further details, it is important to know the relation between the two ranks in food order of each species.

Really, it is entirely difficult to know all the quantitative values necessary for the abundance of all the livings in a certain area. And the composition of fauna differs according to the season of a year, as some species comes in and some goes out when the season changes.

However, if we could find out some fishing method, the catch by which could represent the faunal composition to some degree, by some method of analysis of the data of caught fish, we would be able to know the role of each species in a certain area in a certain season.

Here, YASUDA, by his own idea, tried to analyse the data of experimental fishings done in the coastal area near the Misaki Marine Biological Laboratory of the University of Tokyo, hoping this idea might do something to find the relation between plankton-feeders and carnivorous fish, especially of degree of obstractive role of a certain carnivorous fish against production of a certain plankton-feeders in a certain area.

Our experimental fishings were carried out at three seasons, May 1958, July and October 1957, in Moroiso and Koajiro Bays near the Misaki Marine Biological Laboratory. In each season, several experimental fishings by beach seine and small set net were operated for a few days.

88. **Morphometric comparison of the yellowfin tuna from six grounds in the Indian Ocean.** Kenji KUROGANE and Yoshio HIYAMA (Bull. Jap. Soc. Sci. Fish., 24, 487–494, 1958)

We have reported before about the relationship of yellowfin tuna population from the several waters of the Equatorial Pacific. In the present study we analyse the variations between the samples taken from the six regions within the Indian Ocean, basing upon the morphometric data of 168 specimens collected since 1955.

The following results are obtained from these comparisons. On the yellowfin tuna from the equatorial area of the Indian Ocean, the eastern one generally have a larger head and posteriorly positioned ventral and pectoral fins, and longer fins than those from the western waters. The yellowfin tuna from the south waters of the Lesser Sunda show the great difference with the one from the equatorial area. The former have a shorter head, anteriorly positioned fins than the latter. It may be seen that with respect to the lengths of second dorsal and anal fins, the specimens from the south area of the Lesser Sunda are rather similar to those from the south area of the Andaman Islands, but they have considerably longer fins than the specimens from the Western Indian Ocean.

Accordingly, it is possible by morphometrical comparison that the yellowfin tuna inhabiting in the region of the Lesser Sunda composes a group distinctly separated from

populations of the Equatorial Indian Ocean, and that the one from the Western Equatorial Ocean are somewhat different from the Eastern Equatorial Indian one with some intermingling between each other.

However, with the morphometrical analysis, it is necessary to examine their relationship more exactly from the biological point of view.

89. Morphometric comparison of the albacore from the Indian and the Pacific Ocean. Kenji KUROGANE and Yoshio HIYAMA (Rec. Oceanogr. Works, Jap. 5, [1], N. Ser.: 68-84, 1959)

We have reported before basing on the study of the morphometry of the Western Pacific albacore, that they could be separated into two populations, the Northwest Pacific population and the Southwest Pacific population, and these may widely migrate eastward and westward. In the present study, we have examined the local differences in the Indian albacore, basing our study upon the morphometric data of 125 specimens collected since 1955, and then we have also compared the Indian albacore with the Pacific one.

With the present data available, we may say on the Indian albacore that the ones from the southern areas of the Sunda Islands belong to a common population which the ones from the equatorial areas of the Indian Ocean also belong to.

This study also indicates that there are marked differences in the morphometrical features of the albacore from the Indian and from the Pacific Ocean. In head length and position of some fins, the relative growth rate for the albacore from the Indian Ocean is larger than that for the Pacific fish. Furthermore, the Indian albacore have larger head and more posteriorly positioned fins than the Northwest Pacific albacore. We can see that the Indian albacore is distinct from the Northwest Pacific one from not only their morphometry but also from their separated distributions. From the morphometrical study which shows the rather great difference between the Indian and the Southwest Pacific albacore, we could not consider that these form a homogeneous population. But these difference is not so great as we see in the comparison between the Indian and the Northwest Pacific albacore. Accordingly, they may have intercourse each other to a slight degree through the southern and the northern waters of Australia.

On the yellowfin tuna, racial study basing on the morphometry showed that the fish seem to grow and spawn and at last die in rather confined area. On the contrary, by morphometrical analysis and the tagging experiments, the albacore seem to migrate very widely, transversing the ocean.

90. On the confidence limits corresponding to the estimate obtained by DELURY'S logarithmic catch-effort method. Yukio NOSE (Bull. Jap. Soc. Sci. Fish. 24, 953 956, 1958)

Supposing that the relationship between ln C(t) and E(t) is a simple regression, the author introduced an equation which gives the confidence limits corresponding to the estimate obtained by DELURY's logarithmic catch-effort method. The $(1-\alpha)\%$ confidence limits for N, the size of fish population, are given by the roots of the quadratic equation:

$$N^2[\widehat{k^2} - t_\alpha^2 s^2(c_{22} - \widehat{2k}c_{12} - \widehat{k^2}c_{11})] - \widehat{2k}\widehat{k}\widehat{N}N + \widehat{k}\widehat{N^2} = 0,$$

Where t_α is the value of t at the $\alpha\%$ level for n-2 d.f. and c_{11}, c_{12} and c_{22} are the elements of inverse matrix of the normal equations which determine k and ln(kN).

The author ascertained that the equation is available to check roughly the accuracy

of estimate, by comparing the confidence limits calculated by this method with that obtained from K(t)−C(t) relationship; the source of data being that of Japanese common goby, *Acanthogobius flavimanus* in the northern part of the Tokyo Bay.

91. Some descriptions of the fiishery and biology of the cuttle-fish, *Sepia esculenta* Hoyle, living in Tokyo Bay. Masashi Ishikawa & Eiji Iwai (Bull. Res. Coll. Agr. & Vet. Sei., Nihon Univ. No. 9, 43–51. 1958.)

For a complete knowledge of the life of Decapoden Squid, which is still unknown in detail, this paper is the first of a series to give an account of the cuttle-fish, *Sepia esculenta* Hoyle in Tokyo Bay. In this report, the authors described the fishing methods employed and seasonal changes of catches, and discussed the problems of migration, race and growth of that cuttle-fish. Furthermore, the relations with the environment was studied.

Samples investigated were those materials landed at each fishing port along the coast of Tokyo Bay about all the year round. The study was made on fresh samples as well as those fixed with 10% formalin solution.

Among the results obtained, the notable facts were as follows:

(1) The cuttle-fishes living at the entrance of the bay in winter (60 fathom depth), migrate towards the shallow waters of the coast, especially towards the closure of bay (3 fahom depth) in the early spring.

(2) Although it has been reported by some authors that species *S. esculenta* are divided into two varieties according to the difference of th locular index of the shell, the authors conclude that cuttle-fish in Tokyo Bay may be composed of a single variety.

(3) Mature animals, obtained late in the spawing season, have small body similar to that of animals undergoing growth.

(4) Comparing the specimens caught in spring with those having equal length in autumn, remarkable difference in the locular index is found. Therefore, it is cosidered that the structure of the growth ring and its formation varies due to the different conditions of developmental environments.

(5) Estimated from the salinity at the migration zone, *S. esculenta* may be considered as the species having the pelagic characters.

92. Growth of fin whales in the northern part of the North Pacific. Seiji (Kimura) Ohsumi, Masaharu Nishiwaki and Takashi Hibiya (Sci. Rep. Whales Res. Inst. 13, 97–133, 1958)

The growth of fin whales in northern part of the North Pacific was studied by means of biological data investigated on boards of factory ships from 1952 to 1957.

Owing to the limitation of whaling season within the period from May to September, the breeding season of the northern fin whales is not yet known exactly. Basing upon the data on length of foetuses, it is assumed that the pairing season covers almost the entire year, though the meridian of the year is winter. The gestation period was estimated to be twelve months and the body length at birth was 21 feet in average. The growth curve of foetuses is parabolic and the equation is as follows.

$$L=0.329M^{1.61}$$

Where L is body length in foot, and M is time in month.

The youngest fin whale caught in the northern part of the North Pacific was two and a half years old (five laminations in teeth) and in this stage whales were completely immature. After four years old the animals approach to sexual maturity. By means

of histological examinations male fin whales of which the heavier ones of their testes were more than 2.6 kg were regarded as sexually matured. The largest ones among the sexually immature whales observed were 63 feet long in male and 68 feet in female, while the shortest ones among the sexually mature whales were 55 feet in both sexes. Biological minima in male and female were estimated to be 58 feet and 1 feet respectively. And they attained sexual maturity between four and six years of age in both sexes.

After attaining sexual maturity ossification of vertebral epiphysis began from both ends of vertebral column and accomplished at the area from third to sixth dorsal vertebrae. Female whales attained physical maturity between 22 years and25 years of age, and 24 years in average. The largest ones among the physically immature whales observed were 68 feet long in male and 71 feet in female, while the shortest ones among the physically mature whales were 57 feet in male and 58 feet in female. The average length just physically matured were 62 feet long in male and 66 feet in female. Shrinkage of body occurred after attaining physical maturity.

The maximum life span of fin whales was estimated to be 50 years. In the northern part of the North Pacific, however, it was believed that the physically mature fin whales were very rare.

93. Age study of sperm whale based on reading of tooth laminations. Masaharu NISHIWAKI, Takashi HIBIYA and Seiji (KIMURA) OHSUMI (Sci. Rep. Whales Res. Inst. 13, 135–154, 1958)

For the purpose of revising the size limit for the sperm whales to catch, which is suspected to be too severe when compared wtih that of the other species of whales, it is absolutely necessary to obtain the exact knowledge on the sexual maturity. So, using the materials of the present series of whales collected in the northern part of the North Pacific during the last several years, the authors carried out an investigation on the above-mentioned subject. The results obtained are as follows.

The number of laminations in the teeth is available as the criteria for the age estimation, especially maxillary teeth are suitable for this purpose. Mandibular teeth begin to be defaced several years after birth, and the pulp cavity of the maxillary teeth is closed at over ten years old. The phenomena may be related to the sexual and physical maturity respectively.

The weaning period is estimated to be one year after birth. The body length at this stage is about 21 feet. The male sperm whale attains sexual maturity at the age of four or five (nine or ten laminations in teeth) and the body length at that stage is about 31 feet long and its heavier testis is 0.5 kg in weight. The female sperm whale attains sexual maturity at the next breeding season of the age of four, and the body length at that stage is about 29 feet long. The eruption of teeth have some relation with sexual maturity.

Over the age of eighteen the whale attain physical maturity, and the body length is 52 feet long in male and 36 feet in female.

The number of ovulation in one breeding season is two in an average. That corpus luteum involutes rapidly after parturition but it may not disappear throughout the life.

94. On the adhesive power of the eggs of Ayu, *Plecoglossus altivelis* T. & S. Rikizo

ISHIDA and Yasuo OHSHIMA (Bull. Jap. Soc. Sci. Fish., 24, 896–899, 1959)

As is well known, the eggs of Ayu, after being spawned, attach to gravels of the stream bed by the adhesive membrane, thus tolerating current action. But little work has been done concerning their adhesive power.

In the present study, the authors attempted to estimate the adhesive power of Ayu eggs by means of two different methods. The first method consisted of determining the critical level of current velocity above which eggs could no longer maintain their position, by exposing sample of eggs attached to the inner wall of small glass tubes to water flows of varying velocities. The second method consisted of picking sample of eggs adhering to gravel by forceps one by one and measuring the weight of the gravel which just failed to be raised by being supported by the adhesive membrane.

These two methods gave similar results which were summarized as follows. The adhesive power of Ayu eggs ordinarily lay between 200 and 500 dynes, although it was varied from 180 to 1345 dynes, according to spawners and spawning conditions. Development of eggs was not attended by a change in the adhesive power.

According to the present authors' observations, on the other hand, the current velocity in the bottom layer of the spawning bed of Ayu is generally below 40 cm. per second. The hydraulic pressure which would be given to the egg by this current velocity is estimated to be less than 500 dynes. Hence, the adhesive power may be said to be well adapted to the surroundings.

95. The effect of transplantation on the growth and shell form of the stunted Japanese little necked clam, *Tapes japonica* DESHAYES. Sang CHOE and Yasuo OHSHIMA (Bull. Jap. Soc. Sci. Fish., 24, 616–619, 1958)

In the habitats where shell growth is almost suppressed due to prevailing unfavorable conditions, the little necked clam is rather malformed, thus giving rise to the "stunted" shell. Two groups of stunted little necked clams were obtained from the tidal flat of Ohsaki, Toyohashi City, Aichi Prefecture. One, called here type A, consists of shells formerly transplanted to Ohsaki from the adjacent Muro district, and another, type B, consists of shells native to Ohsaki. Type A of stunted little necked clams had still retained the former form of normally grown shells native to Muro, while type B was distinguishable from normally grown shells by a rounder curvature of the posterior shell margin and a greater ratio of shell breadth to shell length. In both lots, shells of about 2 cm. in length and an estimated age from two (type B) to three years (type A), were transplanted to the clam-bed of Ikawazu, Atsumi Town, in May 1953, and cultured until November for about six months. On the other hand, normally grown little necked clams (about one year of age) native to Ikawazu were used as control. On transplanting and harvesting, the growth and form of the shell were discussed.

The results of the experiment are as follows:

1) After transplantation, the stunted shells of both lots grew as rapidly as the shells which were used as control.

2) After being cultured under good conditions for six months, the stunted shells, especially type B, showed a form of shell with the values of H/L or B/L approached those of normally grown shells.

3) Especially, B/L is a value very significant for the treatment of shell form. In the same age group, the larger values of B/L were observed on ill-grown individuals in both stunted and normally grown shells.

4) The form of shells is largely influenced by environmental conditions of the clam-bed. From the results mentioned in 2) and 3), it is suggested that the form of shells may be used as an indicator of the conditions of clam-bed.

96. On the stock of *Octopus* (*Octopus*) *vulgaris* LAMARCK, on the east coast of Bōso Peninsula, Japan. Jiro TANAKA. (Bull. Jap. Soc. Sci. Fish. 24, 601–607, 1958)

The common octopus, *Octopus* (*Octopus*) *vulgaris* LAMARCK, an important commercial species in Japan, are captured by means of the octopus pot, the hand line and the spear along the east coast of Bōso Peninsula, Chiba Pref.

The writer has made a biological study of the stock of this animal, using the static data of the octopus fisheries based upon the number of specimens taken in Uchiura Bay near Kominato.

The results obtained are summarized as follows;

(1) It is believed that the octopus stock may be composed of two groups, one inhabiting the coast of Bōso Peninsula throughout the year, the other migrating from the north to this district in winter.

(2) The maturation phase of the octopus is shown by hte gonad index (R) as given in the following formula;

$$R = G.W. \times 10^2 / [B.W. - (G.W. + F.W.)],$$

where G.W. denotes the gonad weight,. F.W. the total weight of digestive organ contents, and B.W. denoting body weight.

(3) The breeding season is from March to November, and the biological minimum size is about 40 cm in the male, 40~50 cm in the female.

(4) The monthly increase in the rate of body weight deduced from the body composition and the length-weight relationship is 0.100 during from March to August, 0.180 from June to December.

(5) It may be a reasonable assumption that the immigrant winter group breed during the first breeding season following the migration and that the newly hatched population will increase the available fishing stock from April to July of the following year.

(6) Diminishing rate of the octopus stock is estimated by Dr. TAUTI's method (1956) as 1~2% per day, 5~8% five days, 8~9% ten days and 13% per month. Moreover, the diminishing rate per month is obtained by another Dr. TAUTI's method ('53) as being 11%.

(7) Judging from the diminishing rate caused by fishing, it may be said that the catch efficiency of the octopus pot is greater than that of the hand line.

97. On the seasonal variation of chlor content, transparency and water-color of water of the eel-culture ponds in Tokai district, in relation to the particle size of phytoplankton. Nakaroku NAKAMURA (Bull. Jap. Soc. Sci. Fish. 24, 495–600, 1958)

The thriving of phytoplankton is believed as one of the most important factors directly concerning the daily appetite and respiration of fishes in the eel-culture ponds.

The author had deviced a new method to denote the degree of phytoplankton thriving by the measurements of the transparency using a small china disc, and also, had cleared to specify and notate the water-color numerically by the MUNSELL Color System. Using these new methods, I have studied on the seasonal variation of phytoplankton thriving and its particle size in many eel-culture ponds.

The results so far are as follows:

(1) Chlor content, transparency and water-color of pond waters in five main eel-cultural districts in Shizuoka, Aichi and Mie Prefecture are summarized.
are compared with each other and seasonally by plotting the ratio of chlor content,

(2) Respective characteristics of plankton thriving in the ponds of these districts are composed with each other and seasonally by plotting the ratio of chlor content, transparency and hue value of water-color on the triangular co-ordinates.

(3) While phytoplankton of a minute particle dominates in the ponds in which chlor content of water is higher, relatively macro-sized plankton colony thrives in the fresh-water ponds and especially in the warmer season.

98. On the so-called "Aoko", phytoplankton thriving in the fishculture pond. Nakaroku Nakamura (Aquiculture 6, 72–80, 1959)

The so-called "Aoko", some types of phytoplankters in the fish-culture pond, is considered as one of the most important factors directly concerning the productivity of the pond. However, its production-biological meaning is not yet perfectly evaluated. The author has dealt with some of the commonest species of the "Aoko", the method of measuring the degree of plankton thriving, its meaning at the intensive pond-culture of the eel, and also proper degree of it.

As to the production-biological meaning of the "Aoko" in the pond, the next five points were cited. (1) The value as the origin of oxygen generation in the water on the result of photosynthesis. (2) The value as the food to the various minute animals. (3) The value as the direct food to the pond fishes. (4) The influence to the rising of water temperature. (5) The cause of darkness which is considered as good condition for the pond fishes. In these points, (1) is considered as the most important meaning in the case of eel-culture.

It is obvious that the over thriving of the "Aoko" is unfavorable from many aspects, and the method to control the degree of plankton thriving must be studied in future.

99. On the mingleculture of different species of fish in the eel-culture pond. Nakaroku Nakamura (Aquiculture 6, 127–133, 1959)

Some different species of fish are reared together in most of the eel-culture ponds. This is because the mingleculture is considered as more profitable than the singleculture of the eel. But, the species and amount of fish cultured in the pond are multifarious and interest to it is also different according to the district.

The author has dealt with the meaning of the mingleculture in the eel-culture pond and the present status of it in Tokai district, and also with some considerations about its advantages and disadvanages.

As to the meaning of the mingleculture in the pond, the next three points were cited. (1) Utilization of all layers of pond water. (2) Whole utilization of the given food. (3) Indication of a change for the worse of water.

The carp is cultured together with the eel in the ponds of Toyohashi and eastwards and the crucian and mullet are mingled stil more to them in the westward districts.

From the view point of management, it is evaluated that the mingleculture is more profitable than the singleculture when the price of the eel is not so high. However, the amount of different species of fish should not over the limit in which they do not snatch the food given to the eel. In general, nowadays, annual production of these

fishes is ca. 560-2060 kg. per hectare.

100. On the remedy and prevention of the "Anchor Worm", parasitic copepoda, *Lernaea cyprinacea*, in the eel-culture pond. Shôgôrô KASAHARA (Aquiculture 6, 140-148, 1959)

Parasitism of the "Anchor Worm", parasitic copepoda, *Lernaea cyprinacea*, is one of the most troublesome problems on the pond-culture of the eel in Japan. The females of the *Lernaea* penetrate deep into the skin of the host and it is difficult to kill them without harming the host itself. Then, it is well known that the killing the free swimming larvae is the only way for the extermination of this parasite. Hihero, as the remedy for his purpose, the salt water and the bleaching powder have been used, but in practice these were far from the best method because they brought some unfavorable results in each case respectively.

For the purpose to get the better method to control this parasite, the author has examined a lot of agricultural inseticides and found that "*dipterex*" (the commercial name of o,o-dimethyl-2,2,2-trichloro-1-hydroxyethyl) is the most suitable remedy from the results as follows:

(1) In the *dipterex* solution containing 0.1 ppm of available matter, most of the larvae of this parasite swimming freely or attaching to the fish are killed and in the solution of 0.2 ppm or more, they are eradicated within 3 days.

(2) Some fresh water fishes have much more resistance to the toxicity of *dipterex* than the larvae of the parasite, and the fishes are not only perfectly safe but also can feed in the 0.2-0.5 ppm solution as much as in the case of the blank test.

(3) As to the influence of *dipterex* on the thriving of phyto-plankton in the pond water, there is nothing recognizable as far as the concentration comes up to 10 ppm.

(4) It was evaluated from the results of experiments in the water-tanks and practical fish-ponds that the fish (eel, carp, goldfish, killifish, etc.) could be completely prevented from the parasite by means of repeated treatment of *dipterex* which is put into the water in the concentration of 0.2-0.5 ppm at several times by 2 or 3 weeks interval.

And, aside the above mentioned remedy, an interesting biological fact was clarified by another surveys and experiments that *Mesocyclops leuckartii*, one of the comparatively popular copepoda in the eel-culture ponds at central Chubu district in Japan, had a habit of biting the free swimming larvae of the parasite very actively. Furthermore, a fact that the higher the density of *M. leuckartii* in the water is, the lesser the infection occurs by the parasite was found through investigations performed in many eel-culure ponds. This interrelation seems to show that the parasitism was almost prevented in the water in which a plenty of this copepod was obtained. Therefore, if possible it would be very practical to prevent the parasite so that we can raise this copepod in the fish-culture pond.

101. Effect of water temperature on the growth of sea weed (II). Seibin ARASAKI (Aquiculture 6, 27-33, 1958)

In this report, two problems concerned about the *Undaria pinnatifida* were treated.

1. The 'Naruto-wakame", or a modification of the *Undaria*.

The Naruto Strait is famous for the rapid stream and the big vortices occurring in the time of ebb- or flow-tide, and besides, the shore adjacent to the Strait is famous

as the place producing the special 'Naruto Wakame'. The *Undaria* produced in this area have better quality as food, and some morphological or ecological characteristics different from those found on other places. The principal characteristics are as follows: 1) the young sprouts appear for a longer period covering from January till early July, 2) the vertical expanse of the habitat is wider ranging from the surface to the layer of about 19 m, and 3) the mature fronds produce neutral leaf-lets stretching out on the margin of the sporophyll. Considering on the oceanographical conditions of the Strait, it was suggested that those characteristics were produced by the influence of the peculier conditions, especially the longer duration of water-temperature between 10°-15°C, and the occurence of similar condition from the surface to the deeper layers.

2. The distribution of the *Undaria* along the Japanese shores.

It has been said that the *Undaria*, in the natural habitat, does not distribute at those shores where the water temperaure in annual mean value exceeds lower than 10°C or highr than 20°C. Considering on the monthly change of the temperature, on the other hand, it was suggested that the distribution was checked by the frequent occurrence of the value exceeded lower than 5°C or higher than 25°C. In the same meaning as the 'Indicator of the heat', reported in the Part 1, the author prepared the term 'Indicator of the cold, or Cold Indicator' for the 'frequency or the sum of deviated figures lower than 5°C'.

102. Variability of morphological structure and mode of reproduction in *Enteromorpha linza*. Seibin ARASAKI and Ikuko SHIHIRA (Jap. Journ. Bot. 17, 92-100, 1958)

The phenology of *Enteromorpha linza* was investigated throughout the year at several places near Tokyo. It occurred luxuriantly from autumn to late spring, and within these seasons the mode of reproduction or the form of individual thalli were quite variable from place to place and from season to season. From the mode of reproduction, *E. linza* was separated into two types: firstly it was the pricipal method to make use of sexual reproduction and secondly it was that as exual reproduction is the principal method. In the former type, the ratio of gametophytes to sporophytes was different depending on seasons, and also the same in case of the ratio of males to females. In some areas gametophytes and sporophytes were distinguishable in their shapes or in ecological preferences, but became difficult to distinguish in other areas. In the latter type, the swarmers could usually develop asexually; among them quadri-flagellate swarmers and bi-flagellate swarmers were observed. Also the form of thalli which produced bi-flagellate or quadri-flagellate swarmers was different in some cases, but in other cases they were all similar. The ratio and the ecological preference of the fronds whether producing qudri- or bi-agellate swarmers were variable.

From the observations and experiments it was suggested that the morphological structure and mode of reproduction of *E. linza* could be altered through the influence of environmental conditions. Therefore, students should be prudent in dealing with the taxonomy and life-history of the *Enteromorpha*.

103. The effect of light on gamete liberation in *Monostroma*. Ikuko SHIHIRA (Bot. Mag. Tokyo, 71, 378-385, 1958)

It has been said that the light is an important factor influencing the maturation and liberation of the gamete in *Monostroma*, and that exposure to light following a period of darkness activates the immobile gametes in the cell. This report deals with

a correlation between the light conditions and such activation. The results obtained were as follows:

1) After a sufficient duration of darkness, exposure to ligh appeared to act as trigger agent. 2) The length of darkness necessary to cause liberation became shorter as the 'maturity' of the gamete advanced, and finally occurred without exposure to darkness. 3) When the necessary dark period was interrupted by light its effect was cancelled, and the necessary duration of darkness was not additive. 4) After a sufficient exposure to darkness liberation was induced by 1 sec. irradiation, while insufficient dark periods required longer exposure to light. As the gametes became more 'mature', the 1 hr. exposure to darkness followed by 1 sec. light became sufficient to induce activation, and the quantity of gametes liberated were proportional to the light period within certain limits. 5) Light of wave length shorter than 500 mμ was necessary for liberation. Light of wave length ineffective for liberation had a similar effect to that of darkness, and light of short wave length was effective in nullifying the effect of a preceding dark period.

From the facts above mentioned, it was suggested that in *Monostroma* the reaction which leads to activation of the liberation of the gametes consisted of two parts; the first reaction proceeds at a suitable temperature in the absence of blue light, and when this reaction is completed the second reaction will be induced by momentary exposure to blue light.

104. The characteristics of the thallus of *Monostroma*. Ikuko SHIHIRA (Journ. Jap. Bot. 33, 372–375, 1958)

Various parts of a frond of *Monostroma nitidum* were cut out into many pieces, and their destination were respectively investigated through culture.

Such phenomena as follows were observed: 1) the capacity for maturation was greater in the marginal part, and that for rhizoid formation was higher in the basal part, 2) individual cells have the capacity for independence as if they were unicellular algae, and 3) as a whole, the cells having different capacities in the different parts of the frond were arranged as if the frond were a colonial form.

105. An ecological study of *Undaria pinnatifida* SUR. Part 3. On the effects of light-intensity and water-temperature upon the rate of photosynthesis-1. Yunosuke SAITO (Bull. Jap. Soc. Sci. Fish. 24, 484–486, 1958)

The rate of photosynthesis of *Undaria pinnatifida* was measured by the quantity of oxygen evolved from the materials which were kept in various conditions concerning light-intensity and water-temperature.

Results obtained may be summarized as follows:—

(1) The photosynthesis rate was accelerated with the increase of light-intensity, and reached to a maximum at a intensity correlated to the waer-temperature; i.e. at 10°-15°C at 20,000lux and 5°C at 30,000~35,000 lux.

(2) Under a definite illumination the photosynthesic rate was accelerated with a rise of water-temperature, and the highest value was usually observed at 20°C, but the rate decreased at 25°C. The ratio increased with rise of water-temperature was more gradual under 30,000 lux than under less light-intensity.

106. Absorption and decomposition of the "Paration" in the water by phytoplannokt

and submerged plants. Kazuo SANO and Yoshiyuki MATSUE (Aquiculture 6, 10–13, 1958)

Four species of aquatic plants, *Scenedesmus*, *Spirogyra*, *Najas*, and *Myriophyllum* were tested for their ability to absorb and decompose the insecticide, "*paration*".

The experimental results indicated that "*paration*" was comparatively rapidly absorbed and decomposed by these plants, and the absorption of the plants per weight (in ppm.) was different in each concentration of "*paration*", while the absorption ratio was nearly the same without regard to the concentration concerned. It was also confirmed that the growth of *Scenedesmus* and other aquatic plants were checked by "*paration*" which increased the oxygen consumption.

107. **Oxygen metabolism in eel culture ponds. Part 1. Theoretical fish culture methods based on oxygen metabolism.** Kazuo SANO and Yoshiyuki MATSUE (Aquiculture 6, 43–49, 1958)

So far as is known, the work reported here is the first specifically designed to determine the basic standard of the pond culture of fish such as area, the depth of water, the optimum yield for increasing the yield of fish under some circumstances. Analyses were made on the mechanism of oxygen metabolism considered as the most effective environmental factor, and it was, as the result, ascertained that the depth of water was the most principal to the pond culturalists of fish.

The relationship between oxygen consumption and supply (based on the gaseous transfer into water) in equilibrium led the optimum yield and depth of water as the standard for the pond culture of fish as follows:

$$d \leq \frac{c}{\beta}\left(a - \frac{\alpha}{c} - Z\right)$$

$$\alpha \leq c\left(a - \frac{\beta}{c} d - Z\right)$$

$$\beta \leq \frac{c}{d}\left(a - \frac{\alpha}{c} - Z\right)$$

where d is the depth, α the oxygen consumption defined on an area basis (g/m^2/min.), β the oxygen consumption defined on a volume basis (g/m^3/min.), a the saturated oxygen content (in ppm), c the gas diffusion coefficient, and Z the oxygen content when the consumption and supply are in the balance. (α and β involved oxygen consumption by fish.)

Part 2. The application of theoretical fish culture method for eel culture. (Aquiculture 6, 50–55, 1958)

Putting the values measured in the culture pond of eel into the equations in a previous paper, the same depth of water, when compared with that of the culturalists, was nearly obtained for the optimum yield at the water temperature concerned.

108. **Culturing capacity of the eel-pond from the standpoint of the oxygen balance.** Aritsune SAEKI (Aquiculture 9, 123–126, 1959)

How much eel can be kept in the culture-pond are calculated from the oxygen balance. The oxygen dissolved in the pond-water is supplied from the air and sometimes from the inflowing water. It is consumed by the cultured eel, live or dead organic matter in the water and at the bottom deposits. These supplement and consumption of the oxygen must be balanced and kept at the definite equibirium—its concentration in the pond-water must be kept at 3 ml/1 or 4 3 ppm, in that concentration the eel

should be kept alive and healthy. These balances are estimated and calculated from the culturing capacity of the eel-pond. The followings are the results obtained by some examples.

(1) The pond with the constant inflowing water.

When the area of the pond is 100 m^2, the water depth of its 0.5 m and the volume of inflow water is 27 l/sec., the culturing capacity of this pond is estimated as 2,500-850 kg.

(2) The closed-circulating pond (unchanged water, no filtration and adequate circulation).

When the area, depth and the volume of inflow water are the same as (1), 1,350 Kg of the eel may be cultured.

(3) The general eel-pond.

This pond-water contains much blue-green algae, which supply oxygen in day-time and cosume at night. So at sunset when the algae stop the photosynthesis the oxygen content of the pond-water would be saturated with the air. The eel could be alive at least three hours at the oxygen deficiency. Then this capacity of the pond would be estimated at 3.5-4 kg. per square metre.

These obtained value should be the maximum and theoretical in the eel-pond by our modern technique.

109. Inosinic acid content of foodstuffs. Part 1. Determination method. Takao FUJITA, Yoshiro HASHIMOTO and Takajiro MORI. (Bull. Jap. Soc. Sci. Fish. 25, 147-150 1959)

Inosinic acid (IMP), an alleged tasting substance, was determined by the ion-exchange column chromatographic method on the perchloric acid extracts of several kinds of foodstuff. The method devised by R. BERGKVIST was found to be satisfactory for the separation of IMP from other nucleotides except uridylic acid. The separation of IMP from uridylic acid was attained by changing the column length and composition of eluting agent. It was also found that the time required for the elution of IMP was reducible by increasing the flow rate without any decrease of accuracy. The recovery of the added IMP was satisfactory and ranged from 94.4 to 100.4%.

The fish meat revealed higher values (jack mackerel 853, bonito 739, anchovy 439 mg% on dry basis), whereas the acid was not detected in the foodstuffs of vegetable origin, such as dried mushroom and bean paste. Due to the disturbance of unknown substance revealing an intense absorption in ultraviolet region, the determination of *'sake'* was impossible.

110. Studies on the carotenoids of salmon. Part 2. Determination of muscle pigment. Tsunetoshi KANEMITSU and Hiroshi AOE. (Bull. Jap. Soc. Sci. Fish., 24, 555-558, 1958)

Methods for estimating astaxanthin, the only carotenoid in the muscle of Pacific salmons, have been deviced as follows:

The muscle was macerated and thoroughly extracted with acetone. The pigment was then transferred into petr. ether, chromatographed on a column of cellulose powder, and eluted wtih acetone. The concentration of the pigment in this solution was estimated specrophotometrically. The petr. ether solution of the pigment prepared as mentioned above, or the acetone extract of the muscle also could be used for the measurement of extinction.

In case of fresh meat, there was no appreciable difference among the values ob-

tained by these three methods, the recovery of the added pure astaxanthin being about 95% or more. For the purpose of studying the processed meat, however, it is recommended to adopt the method in which the chromatographic treatment is comprised. By these methods, the astaxanthin concentration was determined on the dorsal, ventral, and caudal muscles of five species of North Pacific salmons, on the muscle of some individuals of the red salmon caught at different stations, and five species were from the same station. Therefore it was ascertained that the muscle of red salmon is the richest in this pigment and the amount of the muscle astaxanthin had no distinct relationship with sex, body weight, body length and fattiness.

111. Trimethylamine oxide in fish muscle. Part 1. The origin of trimethylamine oxide in goldfish muscle. Yoshiro HASHIMOTO and Tomotoshi OKAICHI (Bull. Jap. Soc. Sci. Fish. 24, 640–644, 1958)

To obtain some informations on the metabolism of trimethylamine oxide (TMO) in fish muscle, feeding experiments were carried out and this paper deals with the results on goldfish, *Carrassius auratus.*

Fish were kept in the tank equipped with a filter tank and fed with the diet consisting of carp meat and gelatin with or without addition of TMO.

While goldfish were on the TMO-free diet the muscle was TMO- and trimethylamine (TMA)-free, but it contained a small amount of TMO when fish were kept on the TMO containing diet. In the muscle of fish fed the oxide, TMA also appeared later, and this may suggest together with other evidences that some of TMO is reduced to TMA before being excreted. Moreover, it was observed that TMA was formed in the muscle from ingested choline and none of both bases from betaine. These results may suggest that TMO in goldfish muscle is of the exogenous origin.

Part 2. Trimethylamine oxide in the muscle of eels kept in fresh and brackish water. (Bull. Jap. Soc. Sci. Fish. 24, 645–647, 1958)

To survey the role of trimethylamine oxide (TMO) in the osmoregulation of sea water animals, the effect of diet and chlorinity of medium on the accumulation of TMO was examined by using eels, *Anguilla japonica.*

The eels were divided into two groups and the one kept in fresh water and the other in brackish water (salinity 25%). The fish in both media accumulated the base in the same level as observed on goldfish, while they were kept on the diet containing TMO. The base, however, disappeared rapidly from the muscle, when the fish were fed with the TMO-free diet. The chlorinity of medium exerted no effect on the retention or disappearance of TMO. No appreciable quantity of TMA was detected through the experimental periods, and this may indicate that the metabolic fate of TMO in eels differs from that in goldfish.

Part 3. The origin of trimethylamine oxide in marine fish muscle. Tomotoshi OKAICHI, Masaya MANABE and Yoshiro HASHIMOTO. (Bull. Jap. Soc. Sci. Fish. 25, 136–140, 1959)

Succeeding to the previous works, the similar feeding experiments were extended to a few species of marine fish, globe fish, *Fugu niphobles Jordan* et *Snyder*, filefish, *Monacanthus cirrhifer Teminck* et *Schlegel* and jack mackerel, *Trachurus japonicus Teminck* et *Schlegel.*

A part of TMO in the muscle of globefish and filefish was also confirmed to be of

exogenous origin, but the metabolism of TMO in these marine fishes was found to be significantly different from that in fresh-water fish. The most striking difference existed in the long maintainance of a relatively constant and high level of TMO, even when the supply from the outside was cut off by feeding fish with the TMO-free diet or by keeping them in starvation. In addition, on the muscle of fasting jack mackerel the steady rise of TMO level was observed and this may provide an evidence for the endogenous formation of TMO. Thus, some of TMO in the muscle of marine fish were concluded to be of the exogenous and of the endogenous origin.

112. A rapid color change in the filefish. Tomotoshi OKAICHI, Hisao KAI and Yoshiro HASHIMOTO. (Bull. Jap. Soc. Sci. Fish. 24, 389–393, 1958)

A rapid and marked color change was observed on a filefish, *Stephanolepis cirrhifer Teminck* et *Schlegel*, and four distinct patterns were recognizable. Among twelve fish which ranged from 7 to 9 cm in length and were kept in a tank of 60 litres capacity, there appeared two dominants assuming the slightly dark pattern, whereas the other subordinate indicated no dark pattern. When these dominants were removed from the tank, two dominants newly appeared soon. Thedominants actively drove subordinates away and fought with the other to occupy food and space. The excitement in these activities induced the remarkable dispersion of melanophores. A rapid and extensive aggregation of melanophores resulting in the excitement pallor was also recognized when the fish were frightened violently. It is the most interesting fact that the time required for change of pattern is very short and the shift from one extreme to the other, occurs only in one or two seconds, and this may be the most rapid case so far known on fish.

The similar color change due to excitement was also indicated by a different species of filefish *Rudarius ercodes Jordan* et *Fowler*.

113. Amino acid composition of crustacean muscle proteins. Shōji KONOSU, Shin-ichi KATORI, Teruko AKIYAMA and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. 24, 300–304, 1958)

Eighteen amino acids in the muscle proteins, obtained from three species of crustacean, such as prawn (*Penaeus japonicus*), lobster (*Panulirus japonicus*) and blue crab (*Neptunus trituberculatus*), were determined by the ion exchange column chromatographic method.

The pattern of amino acid distribution in these proteins was relatively uniform, as it was found to be so for fish, whereas some slight differences were observed on several amino acids. The amount of arginine was the most variable, ranging from 6.29% (blue crab) to 9.03% (prawn).

The contents of glycine, alanine, leucine, tyrosine and phenylalanine of crustacean muscle proteins were almost at the same level as those of the ordinary muscle proteins of fish. The crustacean muscle protein showed a higher value of the acidic amino acids, glutamic and aspartic acids, and a lower value of valine, isoleucine, threonine and lysine as compared with the fish muscle proteins.

The muscle protein of the blue crab contained 1.61% of tryptophan, and the value was the highest throughout fishes and crustaceans so far tested in our studies.

114. Amino acid composition of shell-fish proteins. Shōji KONOSU and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. 25, 153–155, 1959)

Eighteen amino acids in the proteins obtained from the edible part of a hard clam (*Meretrix meretrix lusoria*) and an abalone (*Haliotis giantea*) were determined by the ion exchange column chromatographic method.

Although the pattern of amino acid distribution in these proteins was relatively uniform, some slight differences were observed on several amino acids. The protein of abalone was rich in glycine, proline and arginine and poor in leucine, glutamic acid, tyrosine and lysine when compared with that of hard clam. It was also found that the tryptophan content of the hard clam protein is rather high (1.51%).

These shell-fish proteins showed a lower value of valine, methionine, phenylalanine, lysine and histidine than the muscle proteins of fish and crustaceans examined in our previous studies.

115. Studies on the lipid of plankton. Part 2. Fatty acid composition of lipids from Euphausiacea collected in the Antarctic and Northern Pacific Oceans. Masamichi SAIKI, Shin-chen FANG and Takajiro MORI (Bull. Jap. Soc. Sci. Fish. 24, 837–839, 1959)

The lipids of krills, *Euphausia superba* from the Antarctic Ocean and Euphausiacea (*Euphausia pacifica* mixed with a small amount of *Thysanoessa inermis* and *Thysanoessa longites*) from the Northern Pacific Ocean, were examined.

The specimens obtained from the stomach of fin whales in good condition, were extracted with alcohol, acetone and ether, and reddish brown lipids were obtained. The lipid of the former specimen contains less amount of unsaponifiable matter than that of the latter (4.3% and 16.8%).

The fatty acids of the former are composed of 28.2% saturated acids (palmitic 14.4%, myristic 11.9% and the trace of stearic and lauric) and 71.8% unsaturated acids (C_{18} 34.7%, C_{16} 18.6%, $> C_{20}$ 13.6%, C_{14} 4.5% and the trace of C_{12}), whereas the latter are composed of 16.1% saturated acids (palmitic 13.8% and the trace of stearic and myristic) and 83.9% unsaturated acids (C_{18} 52.8%, $> C_{20}$ 15.5%, C_{16} 8.6% and C_{14} 7.0%).

The difference of fatty acid composition between Euphausiacea gathered in the Antarctic and the Northern Pacific Oceans resembles that observed on the blubber oil of fin-whales from the both oceans.

116. Studies on the whale oil. Part 13. The composition of fatty alcohols in the pacific beacked whale blubber oil. Masamichi SAIKI, Shin-chen FANG and Takajiro MORI (Bull Jap. Soc. Sci. Fish. 24, 578–580, 1958)

The quantitative composition of fatty alcohols in the blubber oil of a pacific backed whale, *Berardius bairdii*, was determined.

The saturated and unsaturated alcohols were found to be 38% and 62% respectively, and the latter was proved to be almost entirely consisted of monoethenoid alcohols by the oxidation method with $KMnO_4$ in acetone solution. The fatty alcohols were mainly composed of octadecenol (53%) and octadecanol (27%), and the presence of such a large quantity of octadecanol has not been so far reported on marine animal oils.

In this analysis, alcohols were previously fractionated before fractional distillation by the urea-adduct method, which was very effective to separate octadecenol.

117. Chemical studies on the red muscle ("chiai") of fish. Part 9. Heat coagulability

of red muscle myoglobin and blood hemoglobin. Fumio MATSUURA, Kanehisa HASHIMOTO and Naohisa HARUTA (Bull. Jap. Soc. Sci. Fish. 24, 730–734, 1959)

Heat coagulability of myoglobin ((Mb) and hemoglobin (Hb) of some fish was compared under a fixed condition. Mb or Hb aq. solution was diluted with phosphate buffer to obtain a final concentration of the pigment of 0.075%, and that of the buffer of 0.2 M and a final pH of 6.7. Each 5 ml. of the solution were taken into test tubes, which were then stoppered and kept immersed in ice-water. After heating at various temperatures for 5 min., each tube was immersed again in ice-water, and the remaining pigment in the supernatant was measured spectrophotometrically. Then heat coagulation curve is obtained by plotting remaining amounts of the pigment against temperatures. The noticeable points of the results were as follows.

(1) The positions of heat coagulation curves were different species by species, derivative by derivative, or between Mb and Hb, and the slope of curves for Mb was generally steeper than that for Hb.

(2) On both Mb and Hb, met form was the most heat-sensitive, followed by cyanmet form, while carbonyl and reduced forms were fairly stable.

(3) Each derivative of Hb of a species coagulated at far lower temperatures than the corresponding one of Mb of the species, and this tendency was marked in met and cyanmet forms.

(4) Species specificity of Mb in heat coagulability was evident, which made it possible to divide the fishes tested into two groups; a group having heat-resistant Mb such as tuna, bonito, and swordfish, and another group having heat-irresistible Mb such as yellowtail, saury-pike, and mackerel.

(5) Under the condition employed, however, there was a limit of temperature beyond which no further coagulation of pigment occurred as seen in the case of Mb of bonito or swordfish.

Part 10. A new method for determination of myoglobin. Fumio MATSUURA and Kanehisa HASHIMOTO (Bull. Jap. Soc. Sci. Fish. 24, 809–815, 1959)

A new method for determining myoglobin (Mb) in fish muscle was devised on the basis of the difference in heat coagulability between Mb and hemoglobin (Hb), and the content of Mb in various muscles of some species of fish was measured by this method. Muscle was macerated, extracted with water, and all the pigments in the extract were converted into cyanmet form. The solution was treated with lead acetate and the precipitates were removed. A phosphate buffer of pH 6.7 was added to the filtrate to obtain a final concentration of 0.2 M and the precipitates were removed. The filtrate was heated for 5 min. at a temperature, at which only Hb coagulated completely and Mb remained soluble. Mb in the supernatant was determined spectrophotometrically. Results obtained showed that most of the water-soluble pigments are Mb both in the red muscle (the proportion of Mb to total amount of Mb and Hb was more than 80% in all the fishes tested) and in the ordinary muscle (more than 95% in tuna or bonito), contrary to the previous results obtained by DRABKIN's method which was later found to give too low values in the case of fish Mb due to its characteristic properties such as the low solubility in concentrated salt solution and the instability to lead acetate (in met form).

Part 11. Amino acid composition of tuna myoglobin. Shōji KONOSU, Kanehisa HASHI-

MOTO and Fumio MATSUURA (Bull. Jap. Soc. Sci. Fish 24, 563-566, 1958)

Amino acid composition of the globin, prepared from myoglobin (Mb) of tuna, *Thunnus orientalis*, was determined by the column chromatographic method using ion-exchanger. The following points will be indicated when the results obtained are compared with the amino acid composition of tuna hemoglobin (Hb) or mammalian Mb and Hb in literature.

(1) There are distinct differences between Mb and Hb of tuna, as seen in the case of horse or human, and the general aspect of differences in amino acid composition between Mb and Hb is fairly common to all these species.

(2) There are, however, some differences between tuna myoglobin and mammalian ones. Among monoamino-monocarboxylic acids, alanine is most prevailing in the former, whereas leucine in the latter. Tuna myoglobin contains one cysteine residue, the absence of which has been reported for mammalian Mb. Glutamic acid (11 residues) and basic amino acids (lysine 15, histidine 5, and arginine 2 residues) are considerably poor in tuna myoglobin which is, therefore, far less in free anionic and cationic groups than mammalian myoglobins and rather comparable to mammalian hemoglobins in this point.

118. **Studies on the discoloration in fish meat during freezing storage. Part 1. A spectrophotometric method for the simultaneous determination of ferrous and ferric forms of myoglobin in their mixed solution.** Yoshihiko SANO and Kanehisa HASHIMOTO (Bull. Jap. Soc. Sci. Fish. 24, 519-523, 1958)

A spectrophotometric method was devised which made it possible to follow quantitatively metmyoglobin (MMb) formation in tuna meat during freezing storage. As accepted generally, there are three forms of myoglobin in meat, reduced myoglobin (Mb), oxy-Mb, and MMb. Among those, the former two can be converted readily to carbonyl Mb (MbCO) by standing in CO atmosphere (Case 1). All the derivatives can be converted into MbCO by treating similarly after reducing MMb with sodium dithionite (Case 2), and also into MMb by treating with potassium ferricyanide (Case 3).

If the optical densities at 568 mμ (α-max. of MbCO of fish) of a given Mb solution in Cases 1, 2, and 3 are designated as d, a, and b, respectively, the proportion (x%) of MMb to total Mb in the solution can be calculated by an equation, $x=(a-d)\times 100/(a-b)$. To avoid the autoxidative change of ferrous Mb to MMb in the operation, temperature should be kept as low as possible (near 0°C.) and pH near 7. Accuracy of this method was good, as judged from the results of experiments carried out using the mixtures containing known amounts of pure preparations.

119. **Multiple hemoglobins in fish.** Kanehisa HASHIMOTO and Fumio MATSUURA (Bull. Jap. Soc. Sci. Fish. 24, 719-723, 1959)

Electrophoretic behavior of hemoglobin (Hb) in several species of fish was investigated in a veronal ($\Gamma/2=0.1$, pH 8.6) and a phosphate buffer ($\Gamma/2=0.1$, pH 8.0). According to the results obtained, Hb of swordfish, *Makaira mitsukurii*, is homogeneous in the phosphate buffer but the other Hb's are composed of two (chum salmon, *Oncorhynchus keta*) or three components (yellowtail, *Seriola quinqueradiata*, and rainbow trout, *Salmo irideus*). In the case of carp, *Cyprinus carpio*, we observed two components in an experiment using the veronal buffer and only one component in another experiment

using the phosphate buffer, although the blood samples used in these experiments were not from the same individual.

The heterogeneity of Hb of chum salmon was further examined on the other criteria. There was a critical zone on the solubility curve of salmon Hb, the heat coagulation curves of several derivatives of the Hb were two-stepped, and the Hb was separated into two bands by column chromatography on ion-exchanging resin. All these facts are indicative of the duplicity of the pigment.

120. **Comparative studies on two hemoglobins of salmon.** Kanehisa HASHIMOTO and Fumio MATSUURA (Bull. Jap. Soc. Sci. Fish. 24, 724-729, 1959)

There are electrophoretically two components of hemoglobin in the blood of chum salmon, *Oncorhynchus keta*, and the one which moves faster at the physiological pH was designated as component F and the other component S. They were separated successfully by the method of starch block zone-electrophoresis. Both components prepared thus, were compared with each other in several properties. The pH-mobility curves of component F and S ran approximately parallel, and the isoelectric points were at pH 6.4 (F) and 6.7 (S), respectively. The solubilities of these components in concentrated ammonium sulphate solution differed markedly from each other, component F being less soluble. Component S was far more heat-stable than component F which even matches well the most stable among Hb's of fishes studied so far. Absorption pectra were compared between various derivatives of component F and S, and there were slight but significant differences in the near-ultraviolet region, the spectra of component F locating to longer wavelengths than component S. In chromatographic behavior on ion-exchager, component F moved faster than component S. Thus, the two compoents were found to be more or less different from each other in the various properties.

VI. Forestry

Abstract Nos. 121—141

121. **Influence of electric shock on white grubs. Part 2. Measurement on electric resistance of larvae of *Xylotrupes dichotomus* L.** Kanji TACHIBANA. (Bull. Tokyo Univ. Forests 54, 169-189, 1958)

For the material of this research, the 3rd instar larva of *Xylotrupes dichotomus* L. was very excellent compared with the larva of *Anomara rufocuprea* Motsch. studied in the previous work. To obtain fundamental knowledges, the writer raised this insect in the laboratory.

Xylotrupes dichotomus L. generally completed its one generation of four stages i.e. egg, larva, pupa and adult, in one year. The writer observed the ordinary life cycle of this insect. In the 3rd instar larvae, the breadth of head was 0.90-1.20 cm, length of head was 0.55-0.82 cm, length of mandible was 0.55-0.68 cm, breadth of body was 1.23-2.63 cm and length of body was 5.24-9.35 cm.

The electric resistance of whole body, cuticle, blood and digestive fluid was measured

by the universal bridge using alternating current. Three kinds of electrodes were used properly.

The electric resistance of blood ranged 190-230 ohm and its value increased with growth in the same instar. But on that of digestive and mix fluid, such tendency was not found. The resistance of the former was 110-130 ohm, in the latter was 140-160 ohm and that of enteric fluid was below 100 ohm. The approximate value of the electric resistance of cuticle was 2,000-3,600 ohm and that of whole body was 3,600-4,600 ohm, but in the latter, when the needle-type electrodes were used, the value was only 200-700 ohm.

122. **On the communities of Populus defoliators and their seasonal change.** Chikao NISHIGUCHI. (Bull. Tokyo Univ. Forests 54, 191–198, 1958)

This investigation was carried out through the vegetational seasons of *Populus japono-gigas* (Japanese giant poplar) in Hokkaido, Japan, in 1956 and 57, to analyze the communities of Populus defoliators (in larval stage) and their seasonal change.

Species of dominant and subdominants in each season are given in the following table.

Defoliating Season	Dominant	Subdominant
May	———	*Melalopha anastomosis, Malacosoma neustria*
June	*Malacosoma neustria*	*Lymantria dispar, Erannis sp., Stilpnotia salisis, Noctuidae*
July	*Lymantria dispar*	*Notodonta tritophus, Dicranura spp., Epicnaptera ilicifolia, Biston sp., Noctuidae, Melalopha anachoreta, M. anastomosis*
August	*Melalopha anastomosis*	*Epicnaptera ilicifolia, Dicranura spp., Porthesia similis, Dasychira pudibunda, Trichiocampus populi*
September	*Notodonta tritophus*	*Melalopha anachoreta, Trichiocampus populi, Dasychira pudibunda*
October	*Notodonta tritophus*	*Melalopha anachoreta*

123. **On the virus disease of *Lpmantria fumida* BUTLER. Part 2. On the pathology of polyhedrosis *Lymantria fumida* BUTLER.** Kazumasa KATAGIRI and Ryōnosuke KOYAMA (Journ. Jap. For. Soc. 41, 11–18, 1959)

Observations were made on the virus, and its polyhedra found in *Lymantria fumida* larvae from pathological stand point.

The symptoms of the disease of this host species were generally the same as those in other Lepidoptera species so far reported in lietrature; the infected larvae lost their appetite first and then became flaccid. Just before dying the larvae migrated to the top of the host tree. The length of time from infection to death was on an average eight to nine days.

The tissues susceptible to this virus were fat-bodies, tracheal matrix and hypodermis. Blood was susceptible as well.

This disease occurred first at the time when the most individuals of the insect population reached to the fourth or fifth instar.

The polyhedral inclusion bodies had five or six faces and ranged from 1.5 to 3.5 microns in diameter, though their shapes varied to some extent individually. 0.1 percent sodium carbonate solution was sufficient to dissolve the polyhedra and to reveal their contents in about two minutes.

The process of dissolution was apparently divided into several stages. The process was studied both under an optical and an electron microscope.

The electron micrographs revealed that the polyhedron was dissolved into a number of virus bundles which consisted of three or more virus particles measuring approximately 30×370 millimicrons.

It was found that the polyhedron, the bundle and the virus body had all their own envelopes in which each of them was included.

By an interspecific inoculation test it proved that this virus was strictly specific to *L. fumida* larvae.

124. Notes on Japanese ferns (17-19). Satoru KURATA (Journ. Geobot. 7, 74-77, 113-117, 1958; 8, 14-18, 1959)

From the taxonomical and geobotanical view-points, critical notes are written on such ferns as *Athyrium Kenzo-Satakei* (sp. nov.), *Diplazium virescens, D. virescens* var. *okinawaense* (stat. nov.), *D. virescens* var. *conterminum* (stat. nov.), *D. virescens* var. *Sugimotoi* (var. nov.), *Diplazium yaku-montanum* (new to Honshû), *Dryopteris Dickinsii* var. *Namegatae* (var. nov.), *Polystichum Mashikoi* (sp. nov.), *P. Okanum* (hybr. nov.), *P. ongataense* nm. *Utsumii* (nm. nov.), *P. takaosanense* (hybr. nov.) and *Pyrrosia pekinensis* (new to Japan).

125. On *Asplenium obliquissimum* (Hayata) Sugimoto et Kurata. Jun-ichi SUGIMOTO and Satoru KURATA (Journ. Geobot. 8, 26-27, 1959)

Asplenium obliquissimum (stat. nov.) is clearly distinguished from *A. unilaterale* by its thinner and subhyaline texture of laminae, subcostal sori and wider, ovately lanceolate scales on the rhizome which are not so caudately acuminate at the apex as those of *Asplenium unilaterale*. This fern has been collected in Formosa and Yaku-Island, south of Kyûshû, and now newly found in Honshû, the Kii and Izu Peninsulas.

126. Dendrological studies of the Japanese and some foreign genera of the Thymelaeaceae —Anatomical and phylogenetic studies. Toshio HAMAYA (Bull. Tokyo Univ. Forests 55, 1-80, 1959)

The writer has continued his dendrological study on the Japanese Thymelaeaceae since the preceding paper (1955) was published, and extended, on one side, the scope of study to the wood anatomy and phylogeny, on the other side, the subject to the whole tribe *Daphneae* in DOMKE's sense except the subtribe *Daphnopsinae*. And he examined especially the pentamerous species of *Daphne* and *Wikstroemia* in the Western China.

In the first part, he described the anatomical characters of the wood and bark of the Thymelaeaceous species in Japan. In the second, he took up some important characteristics among the anatomical characters as well as the exomorphic, some of them had been discussed to some extent in the preceding paper. He investigated the directions (or lines) of advance of the characteristics in themselves and the connections between these directions and the relationships of genera. After this investigation or

comparison, he found that most of important anatomical characteristics showed the same or common line of advance represented by the order of genera or sections, while in the exomorphic no common line was found. Also he reexamined the line after the reference of the lines in the whole family and the neighbouring tribes, and, as a result, considered the anatomical characteristics are suggestive of the phylogenetic relationships of the *Daphneae*. Founded on this consideration, a two-dimensional phylogenetic schema was made, and then the writer formed a classification system in the tribe. Also he could clearly determine the coverage of the genera and sections in question.

In the third part, on the basis of his new knowledgements and present opinion, he gave a synopsis of the Japanese Thymelaeaceae in the revised form of the system in the preceding paper, with the distribution maps enriched with new data.

127. **Ueber die Abhängigkeit der Atmungsintensität von Wassergehalt bei keimenden Kiefersamen verschiedener Keimfähigkeit.** Ken-ichi HATANO (Forstw. Cbl. (München) 76, 11/12, 376–384, 1957)

The respiratory intensity of European pine seed (*Pinus sylvestris*) with various germination capacity was compared at the germination time. Before 24 hours from the beginning of swelling, the difference of intensity in them was not yet recognized. O_2 into the seeds with higher capacity was taken up, in parallel with water-absorption, more than in the seeds of poorer capacity after 48 hrs.

The relative lower value of R.Q. at the beginning of swelling indicates the translocation of oil to sugar. The abrupt rise of R.Q. in bad seed example is caused by the micro-organism's action in seed itself.

128. **Anatomical studies on the wood of the Japanese *Pasania*, *Castanea* and *Castanopsis* (With a key to the 22 Japanese representative species of the Fagaceae).** Ken SHIMAJI (Bull. Tokyo Univ. Forests 55, 81–99, 1959)

As a conclusion of the series of the wood anatomical descriptions on the Japanese Fagaceae, five representative species of three genera, i.e. *Pasania edulis* MAKINO, *P. Sieboldiana* NAKAI, *Castanea crenata* S. et Z., *Castanopsis cuspidata* var. *Sieboldii* NAKAI and *C. cuspidata* SCHOTTKY, are minutely described on the wood anatomical characteristics, and some discussions on the relationship of these genera within the family were given, referring to the results obtained on the genera *Quercus* and *Fagus* in the preceding paper. Finally, a key to the 22 Japanese representative species of the Fagaceae based on the wood anatomical characteristics is presented.

129. **Materials for the studies of growth in stands. Part 4. Amount of leaves and production of wood in a young plantation of *Chamaecyparis obtusa*.** Taisitiroo SATOO and Masazi SENDA (Bull. Tokyo Univ. Forests 54, 71–100, 1958)

Studies were made to obtain materials for the analysis of the growth in closed stand in a 28 year old plantation of *Chamaecyparis obtusa* in Tokyo University Forest in Chiba. 28 sample trees consisted of dominant, average, and suppressed trees were taken from three rather small sample plots, and measurement was made on the standing amount of leaves and branches, on current increment of stems, and on the vertical distribtuion of branches and leaves.

The logarithm of the amount of leaves and of branches showed linear relation with the logarithm the diameter breast high. The amount of leaves per 1 ha of woodland

was estimated as about 30 metric tons in green weight and about 13 metric tons in even dry weight. As for the amount of branch per 1 ha of woodland, which is significant as the consumer of the photosynthate in the economy of carbohydrate in woodlands, was estimated as about 16.5 metric ton in green weight, 12 cubic meter in volume, and 0.4 hectar in their surface area.

Stem wood production per tree increased with increasing amount of leaves per tree, and in a ratter larger scope, the amount of stem wood production per tree was proportional to the amount of leaves per tree. The stem wood production per 1 kg of green leaves was 400-600 cc in volume or 160-200 g in dry weight, though it differs according to the conditions of sample plots. If these relations were analyzed in a rather smaller scope, however, among trees with less leaves and with smaller diameter breast high, the stem wood production per unit amount of leaves increased with increasing amount of leaves per tree and with increasing diameter. The stem wood production per unit amount of leaves was smaller in the suppressed trees but there was no difference between dominant and average trees in this respect. The stem wood production per unit amount of leaves increased with increasing height of the tree.

Part 5. Amount of leaves and growth in plantations of *Zelkowa serrata* applied with crown thinning. Taisitiro SATOO, Ken-itiroo NEGISI and Masazi SENDA (Bull. Tokyo Univ Forests 55, 101–123, 1959)

Studies were made on the amount of leaves and its relations with the growth of tree in plantations of *Zelkowa serrata* in Tokyo University Forest in Chitibu, to obtain materials for the analysis of the growth in closed stand. The stands studied were planted on 1912 and a part of which was applied with crown thinning. Measurements were made in 1954 on both parts of the stand, thinned and not thinned. The plot without thinning consisted of 2600 trees per ha and the thinned plot consisted of 983 overstory and 290 understory trees per ha. Sample trees of various dimensions were taken from the two plots, and cubic volume of stems, amount of branches and leaves, and current increment of stems were measured.

The volume of stem, green and oven-dry weight and surface area of leaves, and current increment of the stem showed close connection with diameter breast high, and the logarithm of these values was linear proportional to the logarithm of diameter breast high.

The increment in stem volume per tree showed close connection with the amount of leave per tree, and the logarithm of the increment per tree was linear proportional to the logarithm of the amount of leaves. Stem wood increment per unit amount of leaves was closely connected with the dimensions or degrees of dominance which are represented by diameter breast high, height, and the amount of leaves. And larger and more dominant trees produced more wood by unit amount of leaves. Current increment in stem volume per unit amount of leaves seemed to be larger in the plot which was not thinned.

130. Studies in the growth of young plants of poplar arising from cuttings of different thickness. 1. Seasonal course in dry weight increment. Ken-itiroo NEGISI, Kitokuroo YAGI and Taisitiroo SATOO (Journ. Jap. Forest. Soc. 40, 421–437, 1958)

Growth of young plants of Japanese giant poplar (*Populus japono-gigas*), a clone belonging to Black poplars, arising from cuttings in different size was studied in 1956

and 1957 by following their weight changes in the first season after the planting. Appreciable sprouting of young growth began in mid-April. Dry weight of cuttings decreased in proportion as growth of new parts advanced, because their initial growth was mainly at the expense of materials stored in the cuttings. With the progress of the season, the area of leaf enlarged and the amount of matter produced by assimilation increased day by day. In June the leaves took the place of cuttings as main supplier of materials for growth. Decrease in dry weight of cuttings ceased and conversely began to increase.

The growth of subterranean parts contrasted markedly with that of shoot. A remarkable increase in dry weight of root and cuttings started in September, when the shoot elongation stopped and rate of dry weight increment approached steadily to decline. Gain in weight of roots continued to the end of October.

The course of growth of young poplar in the first year comprised two stages. In the first stage the growth depended on reserves in cuttings, and in the second on assimilate produced by photosynthesis of leaves. The sprouts arising from cuttings of lesser thickness were inferior in growth in the initial stage, and owing to lesser area of leaves they were in a disadvantageous position in growth during the next stage.

131. **Seasonal variation in leaf size of young poplar plants with special reference to height growth.** Ken-itiroo NEGISI, Kitokuroo YAGI and Taisitiroo SATOO (Journ. Jap. Forest. Soc. 40, 499–504, 1958)

Data on variation in leaf size of young poplar growth were presented. Observations were made mainly on Japanese giant poplar (*Populus japono-gigas*), a clone belonging to Black poplars. Leaf area attached to the various parts of the plant in different ages was measured periodically using "dot counting method".

There was a positive correlation between the mean leaf size and mean height of the shoot. With the lapse of the season and the progress of height growth, enlargement in leaf size continued until the beginning of August when the maximum leaf size was observed. Leaf size on the higher shoot was larger in the mean as well as in the maximum than that on the lower one. Stem sprout stood on 1 year old subterranean part expanded larger leaves in early stage of the growth as compared with current year's shoot sprouted from cuttings. In the second year after planting, the leaves on the new stem were larger than those on the new branch elongated from axillary bud.

132. **Hastening the germination of *Pinus densiflora* seeds by gibberellin.** Masasi GOO and Heitarô TSUSHIHASHI. (Journ. Jap. Forest. Soc. 40, 509–511, 1958)

The author has investigated effects of Gibberellin on promoting germination of *Pinus densiflora* seeds which usually show a very low rate unless they are kept under stratification. Three methods were employed here: (1) The seeds were kept in a Gibberellin solution at thermostat of 25°C during the germination test (2) After being soaked in a Gibberellin solution for 3 days at 25°C, washed and subjected to examination (3) Soaked in distilled water for 7 days at 1°C and set on a bed of Gibberellin solutions.

The Gibberellin concentration was divided into 3 levels—10,100, and 1,000 ppm. It was found that 100 ppm and 1,000 ppm concentrations were effective, especially the latter i.e., 1,000 ppm proved to be effective irrespective of the treatment-procedure.

In this case, however, it should be added that though the germination rate was high, germination was somewhat retarded. This delay could be restored when seeds

were soaked in a Gibberellin solution for three days, washed and then germinated. The appropriate time for germination is a matter left for further investigation. It is concluded that a combination of stratification and Gibberellin treatment is proved to be most effective for germination.

133. **Physiological studies of the ripening and germinating processes of pine seeds. (1) Changes of seed proteins.** Masaki KATSUTA (Bull. Tokyo Univ. Forests 55, 125–159, 1959)

This paper deals with the metabolism of proteins and amino acids which occurred in the seeds of Japanese pines during the period from fertilization to germination.

The proteins found in ripe seeds of Japanese black and red pines (*Pinus Thunbergii and P. densiflora*) were glutelin, albumin, globulin and prolamin of which glutelin was most abundant and prolamin very scarce. According to the author's investigation, the main protein of embryo of both pines was albumin while that of endosperm was glutelin. Most of the glutelin and globulin existed in endosperm.

Throughout the period of fertilization, albumin occupied the main part of protein of seed. On the contrary, at the beginning of embryo growth, the quantity of albumin was comparatibly less than that of total protein and other component substances. Comparing the amino acid composition of albumin before and after fertilization, a marked difference was noticed, that is, after fertilization, aspartic acid and serine decreased while arginine, proline and tyrosine increased. Free amino acids such as glutamic acid and amide were formed abundantly in the period of embryo growth. The appearance of abundant free-type arginine at the fertilization period had a close connection with the change of amino acid composition of protein.

Along with the embryo development of seed after fertilization, rapid formation of glutelin, which was very little at the beginning, was noticed. At the end of embryo growth, its synthesis was most active. Globulin formation was also noticed in the latter period. Formation of glutelin and globulin in the endosperm was found to have a close connection with the appearance of seed germinative power. The amino acid composition of protein formed during this period was similar to that of mature seed. In the endosperm of this stage, free amino acids such as amide, proline and γ-aminobutyric acid, etc. decreased rapidly. The rapidity of decrease of these amino acids differed according to the difference of species. This phenomenon, however, was accompanied with the difference of germinative power.

Glutelin in the endosperm was rapidly decomposed immediately after germination, whereas, globulin was not decomposed and albumin very slowly at the same period. Although the amino acid composition of albumin in the endosperm fallen down from seedling was similar to that of the endosperm contained in the seed, the amino acid composition of glutelin and globulin of the former case was quite different from that of the latter case, i.e., arginine, proline, tyrosine, leucine, (valine, serine) decreased and glycine, (aspartic acid) increased in both proteins at the end of germination. These phenomena shows that each protein change independently. In the endosperm, at the beginning of germination, free amino acids such as glutamic acid, leucine, valine, tyrosine, alanine, proline, aspartic acid and glycine appeared abundantly. These amino acids disappeared rapidly in a few days.

Albumin was rapidly formed during the period from embryo to seedling and glutelin was formed in the seedling. Although the amino acid composition of albumin in the seedling was quite different from that of embryo contained in the seed, the amino

acid composition of glutelin of the former case was similar to that of the latter case. The change of the amino acid composition of albumin during the germination period was similar to that of albumin at the fertilization period. Free-type argnine appeared abundantly before germination and amide was formed after germination in embryo.

134. Some field tests of the logging tractor "Fordson" Late. Makoto FUJIBAYASHI, Seihei KATO and Shoji OKAWARA. (Bull. Tokyo Univ. Forests 54, 143-155, 1958)

In the summer season of 1957, the writers had a good chance to carry out a series of field tests of the logging tractor "Fordson F.C." (a 4 t class crawler type tractor made in England) at an operation site in the Obihiro National Forest, Hokkaido.

Some fundamental functions, such as adhesive force of the tracks, tractive effort of the machine, skidding resistance of the load, which are very important for the logging operations, were measured and analized simultaneously.

In this report, some results of the investigation are noted.

(1) State of the runway

For the purpose of the tests, three streches of the runway under practical use, each of which had different kind of the ground conditions, were chosen.

A: forest road, coarse gravel surfaced, dry.

B: minor haulway, ashy clay loam, slightly dry (moisture content 67%).

C: minor haulway, ashy clay loam, wet (moisture content 86%).

(2) Method of measurement

On each runway maximum adhesive force of the tractor just before the beginning of slip, and the average skidding resistance of the load were independently measured by a inserted dynamometer (oil pressure type, self-recording). All these tests were performed at the speed of the first gear.

From the records of the dynamometer the coefficient of adhesion μ and that of skidding resistance μ' were calculated.

(3) On the values of μ, higher figures were obtained on the runway A than on B and C. But the figures are all higher than the generally recognized values, this means that the operation site was favourable for the tractor logging.

(4) On the values of μ', the slightly lower figures were obtained on the runway C than on A and B. μ' means the composed coefficient of skidding pan and that of the loaded timbers partly layed on the ground. The obtained figures show that they are nearly equal to the generally recognized figures of the coefficient of skidding resistance in the case of the direct ground skidding of timbers without any pan.

(5) Maximum possible load to be hauled.

Using the figures above, the maximum possible load in koku (1 koku=0.28 m^3) to be hauled are calculated in regard to the slope of the ground by following formula.

$$W=\frac{M(\mu\cos\theta-\sin\theta)}{\mu'\cos\theta+\sin\theta}$$

W: weight of maximum load to be hauled with skidding pan

M: total weight of tractor including winch

θ: slope in degree of runway

μ: coefficient of adhesion

μ': coefficient of skidding resistance

The results of the concept are shown in Fig. 13. The diagram could be used for the machine operation at the speed of first gear. For the second and the third gear

operation, the corresponding load should be reduced to ca. 90% and ca. 65% respectively.

135. Computation of the tractive force due to the multiple carriage-loads on a cableway. Part 1. Seihei KATO, Masakazu MARUYAMA, Shuenn-Chao WU and Eikichi KOIZUMI (Bull. Tokyo Univ. Forests 54, 157–168, 1958)

Skyline cableways are widely used for timber transportation in our logging operations. The "Bi-cable system', in which multi-span double track cables and an endless tractor cable arrangement are employed, is the most effective type of the cableway for the purpose. Gravity is more or less utilized for the motive power of the cableway of this type. Therefore, it is often necessary to determine numerically the tractive force due to the multiple carriage-loads on the skyline-cable, because we must exactly foresee whether the timbers can be hauled by their own weight only or not, and further more, we must estimate with certain accuracy the maximum amount of the contre-loads (such as yarders, and other heavy materials for the logging operation), which can be carried upwards by the tractive force caused by the descending timber-loads. The authors applied the "influenceline method" for the computation of the tractive force. If we denote the inclination of the curve of the locus of the loaded points on the skyline cable by $\tan\varphi$, the tractive force due to any one load (P) at any point on the cable is given by $P\cdot\sin\varphi$, so that the total tractive force (T_P) due to the multiple loads on the whole track cable can be given by $T_P=P\cdot\Sigma\sin\varphi$. In the same way, the total tractive force due to the contre-loads can be given by $T_Q=Q\cdot\Sigma\sin\varphi$. So that, the effective tractive force $T=T_P-T_Q=P\cdot\Sigma\sin\varphi-Q\cdot\Sigma\sin\varphi$. The authers gave following basic formulae for the computation of the values of $\tan\varphi$ within any one span of the skyline cable.

$$\tan\alpha=\tan\alpha-4s(1+2n)(1-2k)\times\frac{1+6(n+n^2)(k-k^2)}{[1+12(n+n^2)(k-k^2)]^{3/2}}$$

where, $\tan\alpha$: inclination of the span, $s=f/l_0$: Sag-span ratio, f: central sag, l_0=horizontal distance of the span, $n=P/W$: load ratio, P: weight of a carriage load, W: weight of the skyline cable, $k=x/l_0$: coefficient of distance, x: distance of the loaded point from the lower end of the span.

Naturally, above equation shows a curve of higher order, and not suitable for practical drawing, so that it was simplified by substituting an approximate polygon instead of the curve. Then the influenceline for $\sin\varphi$ is also given by a corresponding polygon.

The ordinates of this polygon at both ends of any span carrying a single load are given by the formulae,

$$\sin\varphi_A=\sin[\tan^{-1}\{\tan\alpha-4s(1+2n)\}]$$
$$\sin\varphi_B=\sin[\tan^{-1}\{\tan\alpha+4s(1+2n)\}]$$

and, in the case of carrying a series of multiple loads (numbers of loads$=i$),

$$\sin\varphi_A=\sin\left[\tan^{-1}\left\{\tan\alpha-4s\left(1+\frac{2n}{i}\right)\right\}\right]$$
$$\sin\varphi_B=\sin\left[\tan^{-1}\left\{\tan\alpha+4s\left(1+\frac{2n}{i}\right)\right\}\right]$$

Thus we can easily draw the polygonal influence lines for $\sin\varphi$ on each span.

An example of the influenceline is shown in Fig. 1.

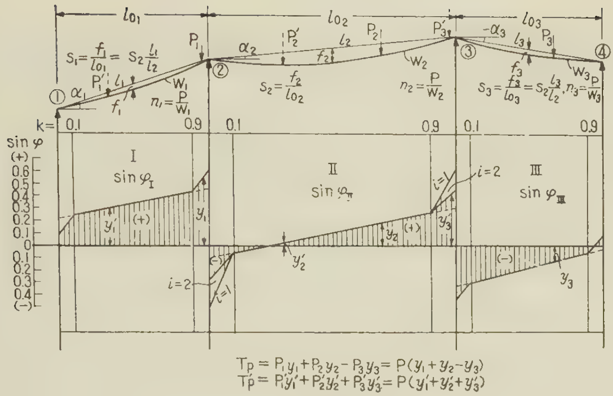


Fig. 1

Part 2. Seihei KATO, Masakazu MARUYAMA, Shuenn-Chao WU and Yoshiyasu TONOMURA (Bull. Tokyo Univ. Forests 55, 181–189, 1959)

The authors carried out some laboratory experiments to prove the reliability of the semi-theoretical formulae for the ordinates of the sinφ-influenceline for a series of multiple concentrated carriage-loads given in report (I).

A light metalic chain, forming a skyline cable of 3500 mm span, was suspended between two fixed points on a wall, on which a series of loads were hanged. These loads were moved simultaneously, and the locus of a load was traced directly on the wall. From this experiment they could make clear the following relation:
in which,

$$y_i = y_o + \frac{y_1 - y_o}{i}$$

y_0: the ordinate of the original unloaded cable at any point.
y_1: the ordinate of the sag of the same point, when a single concentrated load is applied to that point.
y : the ordinate of the sag of the same point, when a series of concentrated loads are applied over the whole span.
i: the number of the loads.

Thus, it was understood that the accuracy of the influenceline concept may be satisfactory.

136. **Planning and design of the logging cableway.** Seihei KATO (Kanehara-shuppan Co. Tokyo, 1959)

The author's studies about almost all fields of skyline cableway construction are systematically arranged in this book, a complete text of the logging six chapters:—

Chap. I. Introduction—A brief history and the present tendency of the logging cableway are described.
Chap. II. Classified logging cableways—15 types are classified and characteristics of each type are clarified.
Chap. III. Theory of the cable—based on the analitical theories of the cable, a series of fundamental formulae for practical culculations are given.
Chap. IV. Wireropes—Manifacturing, mechanical properties and other important things about wireropes are noted.

Chap. V. Planning and design of cableways—methods of planning and location of the logging cableways and practice of designing all parts of a cableway are shown.

Chap. VI. Construction—practice of construction and maintenance are described.

137. Application of hydrograph in expressing the hydrological characteristics of watersheds. Sadao OGIHARA and Isao YAMAGUCHI (Bull. Tokyo Univ. Forests 54, 101–141, 1858)

The hydrograph is one of the most convenient ways to know the runoff condition of a given watershed. But, it is so for individual rain, because the shape of hydrograph varies according to the duration and intensity of rainfall. In this paper the writers show the ways how to utilize hydrographs in expressing the hydrological characteristics of watersheds.

In Part I, S. OGIHARA deals with ordinary hydrographs. In doing so, he selects hydrographs under rains having constant intensities. Then, for each hydrograph the so-called S-curve is derived by adding the ordinates of a series of hydrographs of a kind with their points of beginning separated by the time of the rain duration. S-curves for rains of considerably long durations have flat peaks corresponding to the greatest runoffs. From the shape of the rising part (between the beginning point and the peak) of the S-curve we can estimate how soon the flood peak occurs. An index to be used for estimating times of flood peaks is obtained by calculating the horizontal distance to the inflection point on the S-curve, which is independent from the value of peak runoff, in other words the intensity of rain.

In Part II, I. YAMAGUCHI explains the application value of the principle of the unit hydrograph to the analysis of surface run-off. Basic data are taken from continuous records of run-off and rainfall measured at "Shirasaka" experimental watershed in the Tokyo University Forest in Aichi Prefecture. The watershed area is 88.5 ha. covered with a compartively good forest composed of Japanese red pine mixed with sveral kinds of hardwoods.

The first part of the research deals with a modification of the commonly known

Table 1. Comparison between observed run-off rate and predicted run-off rate

Time hr. min.	Sample 1 1954, Aug. 18		Sample 2 1854, Aug. 31	
	Observed run-off rate m^3/min	Predicted run-off rate m^3/min	Observed run-off rate m^3/min	Predicted run-off rate m^3/min
0	0	0	0	0
20	0.40	0.33	0.90	1.45
40	2.94	3.60	3.32	5.08
1	5.76	6.31	3.18	3.13
20	6.18	5.55	3.28	2.94
40	3.37	2.94	1.98	3.60
2	1.64	1.52	1.10	2.05
20	0.81	0.78	0.54	1.07
40	0.39	0.41	0.23	0.57
3	0.20	0.20	0.11	0.28
20	0.10	0.12	0.02	0.14
40	0	0.04		0.06

unit hydrograph and distribution graph. The latter part of the work is devoted to the application of the unit hydrograph in the analysis of hydrological characteristics of watersheds. Here the writer tries to determine the so-called "equal water travel time" as defined by R. T. Zock, with the aid of the unit hydrograph procedure as used by the writer. Finally, the writer makes comparison between predictive hydrographs obtained by this method and observed hydrographs. The results are shown in table 1.

138. **A report of the scientific investigations of the forest winddomage in 1954, Hokkaido, Japan.** Itizo MINE (Published by Japan Forest Technical Association, 1954)

When unexpected wind havoc had been broken out in Hokkaido 1954, Forest Agency in Japan initiated the scientific investigations to keep a record and research into the cause of the disaster precisely.

The author, as a staff of this investigaion group, made a field survey for more than one month in 1955 and 1956. This is a part of the report of this investigation group. Since the author keeps the data which he attained from his survey on the damage of the Tokyo University Forest in Saghalien, suffered severely from the typhoon which visited in Oct. 1936, he describes at first the outline of this case and then compares it with the damage in Hokkaido in question.

The relation between topography and wind damage is remarkable and the direction of fallen trees was almost reverse to the wind direction. Damages on conifers were more than that of the broad leaved trees. Th majority of damages was root turning and trunk broken, slanted, and tree top broken in order. The smaller the diameter or the tree height, the lesser the damage, and the younger the trees, the lesser damage. In general, while the well managed forest had a considerable resistance, the poorly managed forest was hitted hard. There were quite a number of seedling, but the distribution was not uniform and seedlings were considerably injured by logging, skidding and change in environment. There are bamboo-grass and their growth opress the seedlings of advance growth and the succeeding regeneration is also hampered. However, broad leaved trees grow well on exposd soil causd by wind fallen and logging. For the purpose of observing the future trend, the survey has been made on the wind damaged area about 20 years ago. Judging from the state of forest of this district, it is considered that even if the original forest be of needle leaved, there is a possibility of regeneration into the broad leaved stand. On the contrary, the stand which had been formed after the fallen trees were left as they were, mostly forms conifer stand or mixed stand of conifer and broad leaved trees. The author's suggestion on the future management is described in this paper such as the increase of artificial forest, encouragement on the aid to natural regeneration and silvicultural work, reduction of cutting age, formation of forest having a strong resistance to wind, frost, and snow, disease and insects and regulation of harvesting.

139. **A statistical application of single tree volume table to the "WZP".** Taneo HIRATA (Journ. Jap. Forest. Soc. 41, 209–212, 1959)

For the estimation of stand volume (V) through the "WZP", the author proposed a method combined with a single tree volume table in terms of tree diameter and-height i. e. $v_i=\phi(d_i, h_i)$, by means of the formula written below,

$$V=N\cdot\phi(\bar{d}, \bar{h})\cdot\lambda$$

where N denotes the stem number per hectare, $\bar{d}$ the mean diameter, $\bar{h}$ the mean height,

and λ does the correction factor for the estimation of mean volume E(v) per tree.

The factor λ can be derived by such calculation as follows.

Taking $v_1=\alpha+\beta d^2h$ (combined variable formula, $v_2=d^2h/(\alpha+\beta d)$ (Takata's formula) or $v_3=\alpha d^\beta h^\gamma$ (YAMAMOTO-SCHUMACHER's formula), the expectation of single tree volume E(v) is given respectively as follows, under experimental asumptions that the correlation coefficient between tree basal area (g_i) and -height (h_i) is nearly equal to 2/3, and the coefficient of diameter (C_d) is about two times as large as that of height (C_h) in plantations.

$$E(v_1)\doteqdot\alpha+\beta\bar{d}^2\bar{h}\cdot\left(1+\frac{5}{3}C_d^2\right)$$

$$E(v_2)\doteqdot\{\bar{d}^2\bar{h}/(\alpha+\beta\bar{d})\}\left(1+\frac{5}{3}C_d^2\right)$$

$$E(v_3)\doteqdot\alpha\bar{d}^\beta\bar{h}^\gamma\cdot\lambda$$

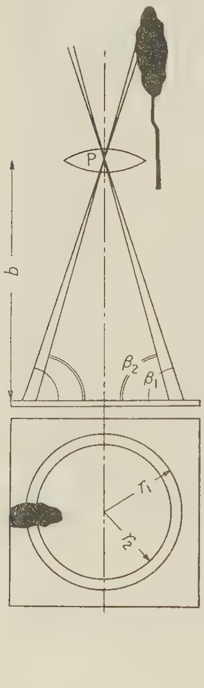
with $\lambda\doteqdot\left\{1+\frac{\beta}{2}(\beta-1)C_d^2\right\}\left\{1+\frac{\gamma}{8}(\gamma-1)C_d^2\right\}\left\{1+\frac{\beta\gamma}{3}C_d^2\right\}$, or $\lambda_R\doteqdot1+\frac{5}{3}C_d^2$ in case β and γ is rounded respectively by 2 and 1.

Thus the stand volume can be estimated by such method more speedily than by a conventional one summing up each tree volume, and the accuracy of this method was warranted by some practical comparisons with the latter case.

In case estimations of tree volume by diameter class are postulated, we have only to divide the total volume estimated at first stage by the above method, proportionally to the basal area by diameter class.

Making use of the range of diameter, σ_d can be easily estimated to calculate the λ.

The "WZP" should be used being combined with above method, because factors in the formula: $V=N\cdot\phi(\bar{d},\bar{h})\cdot\lambda$ can be estimated with efficiency by the "WZP" of two kinds (horizontal and vertical) and calipering nearest trees at sample points.



140. A photographic estimation of stand mean height. Taneo HIRATA (Journ. Jap. Forest. Soc. 41, 178–179, 1959)

For the conometeric estimation of the stand mean height by the auther, he made use of a camera (see Fig.), where an observer at sample point has only to count the number of trees, tops of which are projected within the circle of constant radius printed on the pint glass or drawn on the picture printed. The estimating formula for the conometeric mean height is given by

$h_c=(100\sqrt{k\,B_c/N})\tau+a$ in meter.

where h_c denotes the mean height, k the constant determined by the angle (β) of conometer, B_c the mean of the conometeric tree count, N the stem number per hectare, τ the correction factor for the slope (θ) area ($=E\{1-tg^2\theta\cot^2\beta\}^{\frac{3}{4}}$), a the eye height in meter.

141. **Precisions of variable plot methods (WZP.)** Taneo HIRATA (Bull. Tokyo Univ. Forests 54, 1-17, 1958)

On the assumption resulted by the author from theoretical and empirical views that the variance of the tree count in variable or invariable plot methods is nearly equal to the mean i.e. the distribution of the tree counts can be approximated as Poisson type in forestry practice, precisions of sampling method by point (Bitterlich, Hirata), line (Strand) and plot were compared one another; it resulted from theoretical and practical points, that the point sampling was definitely more precise and expedient than others.

VII. Forest Products

Abstract Nos. 142—158

142. **The influence of temperature on creep of wood by bending test.** Kakuichi KITAHARA and Noboru OKABE (Journ. Jap. Wood Research Soc. 5, 12-18, 1959)

When temperature is constant, the relationship between time and creep has been reported by several researchers as follows,

$$\delta_c = At^N$$

where δ_c is the creep deflection, t is time and A and N are the constants. Creep deflection (δ_{c-t}) at a certain time t, however, is indicated by the following formula when temperature changes,

$$\delta_{c-t} = k\ exp(\alpha T)$$

where T is the temperature and k and α are the constants.

Experimental formulae of creep deflection.

Load (Stress)	Temp λ (°C)	Exp. formula
5.5 kg (148.5kg/cm^2)	5	$\delta_c=1.9\,t^{0.27}$
	10	$\delta_c=2.3\,t^{0.32}$
	20	$\delta_c=4.0\,t^{0.34}$
	30	$\delta_c=8.2\,t^{0.34}$
	50	$\delta_c=28.1\,t^{0.35}$
	70	$\delta_c=105.4\,t^{0.37}$
3.5 kg (94.5kg/cm^2)	20	$\delta_c=1.4\,t^{0.27}$
	30	$\delta_c=2.3\,t^{0.31}$
	50	$\delta_c=7.4\,t^{0.35}$
	70	$\delta_c=30.2\,t^{0.35}$
1.75 kg (47.5kg/cm^2)	50	$\delta_c=2.6\,t^{0.32}$
	70	$\delta_c=8.9\,t^{0.32}$

α has no relationship to fiber stress and time, as shown in Table, but is a function of time (t) and fiber stress (σ).

Then, $k=(p\sigma^2+q\sigma+r)t^n$

where p, q, r and n are the constants.

Creep deflection, consequently, is indicated by the formula below;

$$\delta_c=(p\delta^2+q\sigma+r)t^n exp(\alpha T).$$

Velocity of creep is presented as follows;

$$V_c=n(p\sigma^2+p\sigma+r)t^{n-1}exp(\alpha T).$$

143. Studies on surface roughness of wood. Part 2. A simple apparatus for determining surface roughness and its applicable conditions. Yôji KIKATA (Journ. Jap. Wood Reseach Soc. 4, 216-219, 1958)

The results of the measurement of wood surface roughness under several conditions by using simple apparatus are indicated in this report.

1. The Radius of Ball Point. When the radius of a ball point is too large, the tracing needle (ball) can not touch the bottom of a small valley of the surface, and the profile curve obtained is inaccurate. On the other hand, when the radius is too small, the tracing needle (ball) falls into such small valleys as wood pores or other cavities due to the workmanship in construction, which are smaller in comparison with opening on the surface to be measured. The smaller openings are eliminated from the evaluation of the "maximum height roughness" of the derived profile curves, is difficult as they make a continuous measuring.

These difficulties can be solved by assuring a definite amount of errors in advance from the following two formulae (a) and (b) (Figs. a, b).

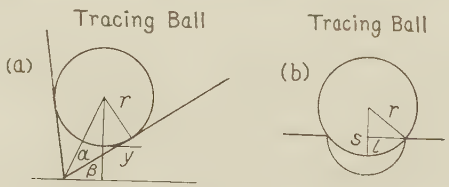


Fig. 2. a) Error (y) Due to Too Large Point Radius (r).
b) Increased Roughness Value (S) Due to Smaller Opening ($2e$) On Surface Expressed by an Arbitrary Point Raudius (r)

$$y=r\left\{\frac{\sin(\alpha+\beta)}{\sin\alpha}-1\right\}, \qquad (a)$$

$$s=r\left\{1-\sqrt{1-\left(\frac{l}{r}\right)^2}\right\}, \qquad (b)$$

The former indicates error due to a too large point radius, and the latter indicates an appreciable roughness value at the smaller opening of a valley when an arbitrary point radius is used.

Under some bad conditions in this experiment, the errors in (a) are ranging $5\sim10\,\mu$ at the mean roughness of $50\sim100\,\mu$ ($2\alpha=160°$ and $r=600\,\mu$), and the appreciable roughness values in (b) are found to be $25\sim76\,\mu$ when vessels of Yachidamo

(openings=250∼400 μ and r=300 μ) are measured.

A suitable point radius should be determined by taking into consideration the kind of measured surface roughness and other conditions.

In this experiment, the point radii of the balls are in any cases suitable for continuous measuring.

2. Pressure of Ball Points. Wood is crushed under a large pressure of ball points. Depths of indentations on several species of wood are tested, from which it may be said that the values of pressure of points, especially on a low value, are suitable for measuring, except the higher value for the soft spring wood of Sugi.

144. Studies on the impact withdrawal resistance of nail in wood. Part 2. Shitomo MAMADA (Jour. Jap. Wood Reserch Soc. 5, 27-31, 1959)

A study on the impact withdrawal resistance of nail was developed using the same apparatus as in the previous report (Records of Res. in the Fac. of Agr. Univ. of Tokyo, No. VII, 77-77, 1958). In this study the energy for pulling out the nail has been investigated in more detail.

That is, the energy value of obtained by this apparatus is apparently the sum of an energy for blowing off the attachment (Ea) and that for pulling out the nail (Ew). The relation between Ea, Ew and the reading of the scale on the testing machine is shown in Fig. 1.

The test results are as follows:

1. The impact withdrawal resistance increased as the depth of driving was increased which was characterized by nearly straight line.

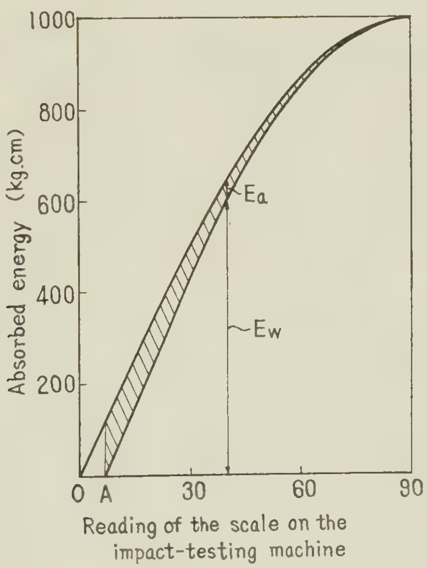


Fig. 1.

2. The relation between diameter of nail and impact withdrawal resistance was given as the following empirical formula.

$y=106.5e^{0.245x}$ ....SUGI (*Cryptomeria japonica*)

$y=84.8e^{0.305x}$ ....HINOKI (*Chamaecyparis obtusa*)

Where y is the impact withdrawal resistance, and x the diameter of nail.

3. The impact withdrawal resistance increased as the specific gravity of wood was increased which was characterized by nearly straight line.

145. Studies on laminated woods with sawn boards. Part 5. On the durability of glue joint of urea resin adhesives filled with wood flour in the laminated woods with sawn boards of sugi (*Cryptomeria japonica* D. Don). Hisashi FUKUI and Shinji HIRAI (Journ. Jap. Wood Research Soc. 4, 195-200, 1958)

The authors prepared laminated woods with sawn boards of sugi by using urea resin adhesives filled with wood flour and unfilled straight resin. Grain sizes of wood flour are three kinds, those are 80∼100 mesh, 120∼140 mesh and less than 200 mesh, and content of wood flour is changed in three, that is 5, 10 and 15%. Dimensions of prepared laminated woods are 1000×66×80 mm. Test pieces for adhesive strength of tensile-shearing type were taken from laminated woods, and some of them were exposed to four accelerating conditions and the others were put in normal dry condition. From the results of adhesive strength test the following observations are obtained.

1. When the content of wood flour is 5∼10% of liquid resin, the durability of the joint of filled adhesives increases a little compared with that of unfilled straight resin.

2. But when it amounts to 15%, there is rather a little decreasing of durability.

3. As for grain size of wood flour, it seems to be that the smaller the greater the durability is.

146. Studies on weight growth of forest trees. Part 6. *Chamaecyparis obtusa* ENDLICHER of the Tokyo University Forest in Chiba. Shinji HIRAI (Bull. Tokyo Univ. Forest, 54, 199-217 1958)

Three sample areas in an artificial stand of *Chamaecyparis obtusa* ENDLICHER of 28 years of age were chosen at the Tokyo University Forest in Chiba Prefecture. Twenty eight sample trees were taken from the areas and the distribution of bulk density of wood (calculated from weight in oven dry and volume in green) within the stem of each sample tree was examined. From these results the weight increments of individuals and the weight produces of the areas were calculated. Following results were obtained.

(1) Most of individual frequency curves of bulk density are left-inclined and the total frequency curve is also left-inclined. The degree of left-inclination is larger compared with the species about which the author reported previously. The value of coefficient of variation is rather low.

(2) The state of distribution of bulk density varies individually to some extent, but the following general tendencies are recognized. The wood is heavy through the center of tree. The heaviest wood appears at the top of tree most frequently and at the tree base secondly. As to the vertical change along the center of tree, the wood is heavy at the tree base, soon becomes lighter in the middle part and turns heavy towards the tree top. As to the horizontal change at the height 0.3 m from ground, the wood becomes lighter from the center until 25 years of age successively, and then

ascends a little in the outest part, that is 28 years of age. The range of the lightest wood develops diagonally from the outest part at the height 3.3 m downwards into the inner part.

(3) Seventeen sample trees indicate the maximum current annual increment of volume at 20 or 25 years of age. Fifteen of them indicate the maximum current annual increment of weight simultaneously, and the other two have not yet attained to the year of the maximum of weight at 28 years of age. Six sample trees indicate also the maximum average increment of volume at 25 years of age and all of them indicate the maximum of weight at the same age. Generally speaking, the weight increments of the samples are mostly affected by their volume ones. The value of increment percent is higher in volume than in weight in the earlier stage, but in several instances it turns contrary after 25 years of age. This tendency is rather clear in inferior trees.

(4) The standard bulk density of the individuals, which is obtained by dividing the total weight increment by the volume one, is 381~489 kg/m^3, and the mean is 418 kg/m^3. The relation between the total increment in volume and the standard bulk density is not clear. But roughly speaking, there may exist a tendency that the smaller the total increment in volume is, the larger the standard bulk density is.

(5) The total weight produces per 1 ha of these researched sample areas amount to 88,119 kg, 57,337 kg and 59,771 kg respectively, and the mean is 66,911 kg. The average annual produces are 3,147 kg, 2,048 kg and 2,135 kg respectively, and the mean is 2,390 kg. The compilation of the result is shown in the table.

Table. Volume and weight produces of stands (per 1 ha).

No. of sample area	Number of stems	Volume		Weight	
		Total produce m^3	Average annual produce m^3	Total produce kg	Average annual produce kg
1	5941	211.73	7.76	88,119	3,147
2	5592	137.32	4.90	57,337	2,048
3	5174	149.17	5.33	59,771	2,135
Ave. with weight of area	5556	162.40	5.80	66,911	2,390

147. Studies on indication of wood quality of forest trees. Part 1. Indication of several qualities of the wood of *Abies firma* Siebold et Zuccarini. Shinji Hirai and Hikaru Yajima (Bull. Tokyo Univ. Forest, 54, 219–236, 1958)

The authors tried to express numerically several qualities of wood so as to represent qualities of the whole bole of individual tree. Two sample trees of *Abies firma* Siebold et Zuccarini were taken from a natural stand in the Tokyo University Forest in Chiba Prefecture. Sample discs and cants were cut off in different heights from ground. Measuring methods are as follows.

(1) Unevenness of annual ring width.

Two methods were adopted.

a) Standard deviation of ring width.

Average ring widths were measured with each five rings which were marked successively from the pith to the bark in four directions on each disc. Standard deviations of average ring width were calculated with each direction and the representative value of a disc was gained by averaging values of four directions with weighing the represented area of each direction. In the same way, the representative value of the whole bole was gained by averaging values of discs with weighing the represented volume of each disc. Proportional values of standard deviation to ring width were also calculated.

b) Successive difference of ring width.

Means of average ring width of each age grade of five years were calculated, and then successive differences of means as to age grade were calculated. Thus the representative value of a disc was gained by averaging simply the difference values. The representative value of the whole bole was gained by averaging values of discs with weighing the represented volume of each disc. Proportional values of successive difference to ring width were also calculated.

(2) Wavines of annual ring.

Measurements were taken with edge grain faces of four sample cants in each different height from ground. Measuring method is shown in Fig. 6. Waviness of a ring on a face was expressed as follows.

$$z=\frac{|x_2-y_2|+|x_3-y_3|+\cdots+|x_9-y_9|}{8}$$

The representative value of a face was gained by simply averaging measured values of several rings, and the representative value of a log was gained by averaging values of four faces with weighing the area of each face. Then the representative value of the whole bole was gained by averaging values of logs with weighing the volume of each log.

(3) Knot percentage.

Measurements were taken with edge grain faces of four sample cants in each different height from ground. The total areas of knots on each face were measured by a planimeter, and the representative value of a log was indicated by [Σ (Total area of knots in four faces)/Σ (Area of face)]$\times$100(%). Then the representative value of the whole bole was gained by averaging values of logs with weighing the volume of each log.

(4) Percent of imperfect heartwood.

Sample tree No. 2 has an imperfect heartwood of dull pale brown colour. This seems to be equal to the "Dunkler Kern" which was described by H. KNUCHEL (1947).

Percent of imperfect heartwood was measured with sample discs, and the representative value of the whole bole was gained by averaging values of discs with weighing the represented volume of each disc.

(5) Percent of summer wood.

Percent of summerwood was measured only with discs of 1.3 m from ground.

Obtained results are shown in the following table.

Table. Wood qualities in total bole.

Item		Sample tree No. 1	Sample tree No. 2
Unevenness of ring width	Standard deviation of ring width (mm)	1.556	0.951
	Proportional value of the former item	0.556	0.307
	Successive difference of ring width (mm)	0.592	0.493
	Proportional value of the former item	0.220	0.161
Ring waviness (cm)		0.714	0.432
Knot percent (%)		0.919	0.622
Percent of imperfect heartwood (%)		—	45.6
Percent of summer wood (Measured only at 1.3m from ground) (%)		28.27	35.28

Part 2. Percentage of false heartwood and unevenness of annual ring width in the wood of *Fagus crenata* Blume. Shinji Hirai (Bull. Tokyo Univ. Forest, 54, 237–243, 1958)

Table 1. Number of annual rings, average ring width and percent of false heartwood.

Trivial name	"White"			"Red"		
	Max.	Min.	Ave.	Max.	Min.	Ave.
Diameter of log without bark (cm)	64.0	36.0	49.4	76.0	40.0	54.6
Number of annual ring	192	74	129	225	115	180
Average ring width (mm)	3.99	1.25	2.02	2.41	1.13	1.54
Percent of false heartwood (%)	29.3	2.2	11.5	59.6	6.4	33.6

Table 2. Unevenness of ring width.

No. of log	Trivial name	Diameter at butt end (cm)	Number of annual rings	Average ring width (mm)	Standard deviation of ring width (mm)	Proportional value of the former item	Successive difference of ring width (mm)	Proportional value of the former item
1	"White"	68	136	1.78	0.47	0.26	0.37	0.21
2		58	113	2.49	0.66	0.26	0.35	0.14
3		51	125	1.98	1.28	0.64	0.39	0.19
4		60	109	2.67	1.25	0.47	0.67	0.25
Mean of "white"				2.23	0.92	0.41	0.47	0.20
5	"Red"	61	202	1.46	0.40	0.27	0.33	0.22
6		67	254	1.29	0.70	0.54	0.40	0.31
7		55	241	1.09	0.41	0.37	0.28	0.26
8		64	258	1.19	0.41	0.35	0.33	0.27
Mean of "red"				1.26	0.48	0.38	0.33	0.26

In the southern district of Hokkaidô, beech wood (*Fagus crenata* BLUME) is conventionally devided into two kinds with the trivial names, "white" and "red", by its bark appearance and occasionally also by the amount of false heartwood. The author has formerly deducted that the difference is not clear botanically and that it is reduced to the growth condition concerned to habitat or age. In this report the author studied the percent of false heartwood and the unevenness of annual ring width as indicators of wood quality by the method almost similar to the previous report. Obtained results are as follows.

(1) The values of the number of annual rings, the average ring width and the percent of false heartwood measured on twenty-one logs of "white" beech and thirty-one logs of "red" beech are listed in Appendix, and the summarized result is shown in Table 1. "Red" has a smaler value of average ring width than "white", but has a larger value of percent of false heartwood.

(2) Generally "red" is higher in age than "white", and the shape of false heartwood in the cross section is mostly near circle which is suggestive of normal heartwood formation.

(3) Measurements of the unevenness of annual ring width are shown in Table 2. The absolute value of the successive difference of ring width are smaller, but in the contrary the proportional value of the successive difference of ring width is larger in "red" than in "white". It seems to be rational to indicate the wood quality with the last factor in the meaning of thinking over partial variance.

Part 3. Unevenness of annual ring width in the wood of *Abies firma* SIEBOLD et ZUCCARINI from Kido district, Fukushima Prefecture. Shinji HIRAI (Bull. Tokyo Univ. Forest, **55**, 191-209, 1959)

The unevenness of annual ring width as a factor of wood quality was studied dealing with the wood of *Abies firma* SIEBOLD et ZUCCARINI from a natural stand in Kido district, Fukushima Prefecture. Four sample trees, include two of so-called "himomi", meaning dry fir, and two of so-called "mizumomi", meaning wet fir. The researching method was almost similar to that of 1st report.

Observations are as follows:

1) Absolute values of the standard deviation and successive difference of ring width are large near ground (0.4 m from ground), decrease abruptly towards 2.3 m, and hold few fluctuations through the upper part of bole. There seems to be a little decreasing of values at the top part. The proportional values indicate almost similar variations as the absolute values, but the proportional value of successive difference of ring width shows some descent between 2.3 and 6.3 m from ground.

2) Comparing values of hill side and down side in cross section of bole, no evident relation is extracted, but in the lower part of bole there are some possibilities that the proportional value of successive difference of ring width of down side is smaller than that of hill side, or that the proportional value of successive difference of ring width of the side, in which the average ring width is larger than that of the opposite side, is smaller. This tendency is no more evident above 6.3~10.3 m from ground.

3) As to the relations between the average ring width and the four factors within the range of 2.3~18.3 m from ground, where the fluctuations of values are comparatively few, there are no evident tendencies, but roughly speaking, it seems to be that the larger

the average ring width is, the more the absolute values are, and on the contrary the smaller the proportional values are.

4) There is no parallel ranking as to four factors. Based upon the principal factor, that is, the proportional value of successive difference of ring width, the trees are ranked as follows: No. 3, No. 1, No. 4, No. 2.

5) The so-called "mizumomi" (wet) has larger four factors than "himomi" (dry), discarding the sample tree No. 4, because of its abnormality of wood formation and lack of discs of upper part.

6) As shown in table, the samples from Kido district, Fukushima Prefecture have larger proportional values of successive difference of ring width than those from Chiba Prefecture on the average.

Table. Comparison of fir woods from Chiba Pref. and Fukushima Pref. as to unevenness of ring width.

Locality	No. of sample tree	Standard deviation of ring width mm	Proportional value of the former item	Successive difference of ring width mm	Proportional value of the former item
Tokyo Univ. Forest in Chiba pref.	1	1.556	0.556	0.592	0.220
	2	0.951	0.307	0.493	0.161
Kido district, Fukushima Pref.	1	1.01	0.394	0.66	0.253
	2	1.00	0.383	0.76	0.291
	3	0.84	0.344	0.53	0.218
	4	0.95	0.536	0.82	0.255

148. Wood quality. Shinji HIRAI (A Report of the Scientific Investigations of the Forests Wind-Damaged in 1954, Hokkaido, Japan, 291–330, 1959)

This part deals with the wood quality of damaged trees from wind in 1954.

(1) Sawing to detect compression failure and cross break on sawn surfaces of damaged trees.

Eight specimen trees of *Abies sachalinensis* MASTERS were taken from a wind-damaged site at 166 D, Ufun, near Asahikawa. All logs were sawn into boards of 5 Bu in thickness (1 Bu≒3 mm) from windward to lee side, and then boards were planed. Compression failure and cross break due to wind pressure were examined on the surface of each sawn board, and their number, position and length (depth) were measured respectively.

i. Generally speaking, the type of compression failure appears on the compression side of the bole, that is lee side as to wind pressure, and the type of cross break exists on the tension side, that is windward, but occasionally two types are found on either side. In some instances, there are splits parallel to grain at the top of cross break due to horizontal shear. Some abnormal breaks which cross fiber in the middle part of board in considerable length are also found.

ii. Compression failure and cross break appear remarkably in six within eight specimen trees. It is considered that they exist generally whether the tree was uprooted

or stem-broken, and their existence in undamaged standing trees is also expected.

iii. Compression failure and cross break distribute in upper and lower parts from break point of stem-broken tree, and in an instance they attain to over 9 m above the point.

(2) Density measurement of wood as indication of quality deterioration in relation to conditions after wind damage.

Many specimens were taken from trees or logs at various conditions after wind damage in many places under the superintendence of Asahikawa, Kitami and Obihiro Regional Forest Officies in summer, 1956. Specimens were also taken from undamaged standing trees in the same place for comparison. Several discs were cut from each specimen, and test pieces for density measurement were taken from discs. Density of wood were determined by oven-dry method. Though three important conifers in Hokkaido, i.e. *Picea jezoensis* CARRIÈRE, *Abies sachaliensis* MASTERS *and Picea Glehnii* MASTERS, are comprised in the measurement, the main objective of the study is placed on *Picea jezoensis* due to the number of specimens.

i. The density of sap wood of fallen part of tree, containing uprooted stem, fallen top part of stem-broken tree and stored log, is smaller over 10% than that of undamaged standing tree on the average in various instances. This is also recognized by the fact that the density of sap wood is ordinarily higher than that of adjoining heart wood in the undamaged normal tree, but there is a contrary tendency in the fallen part of tree. By exterior examination of discs, there is usually discoloration or slight rot in the fallen part of tree and occasionally with worm or borer's holes.

ii. Similarly, the standing bole of stem-broken tree has also lower density in sap wood than that of undamaged standing tree.

iii. The deterioration of density of sap wood in the stored log in timber-yard may also be recognized.

(3) Observations about the quality deterioration of wood of standing tree at wind-damaged site.

Several types of the quality deterioration of wood are listed, and some observations about them are described.

i. R. TRENDELENBURG (1936) reported on the formation of knurl upon compression failure area of wind-damaged bole, and F. MERGEN and H. I. WINER (1952) have also reported on the similar formation of pitch pocket and knurl of bole. The author searched the defect at wind-damaged sites in 1936, but he couldn't nd any proof of it. Sometimes elevated streaks are found on the surface of bark of standing tree, but there is no change beneath the bark.

ii. The author indicated the unevenness of ring width due to the wind damage in 1936, and also detected the enlargement of ring width and the formation of remarkable compression wood after the wind damage in 1954.

iii. The frost shake of standing tree at wind-damaged site, was observed. It is not clear that the frost shake appears primarily due to the extreme change of circumstances by wind damage.

(4) Miscellaneous observations.

i. Investigations were carried out on the observed split length with bark and the measured one without bark mainly as to stem-broken trees at Tomakomai and Shikotsu. The tendency is different by species, that is, the two lengths don't change markedly by *Abies sachalinensis*, but the actual split is extremely longer than the observed

split by *Picea jezoensis*, and sometimes the difference attains to 3 m. The difference is clearer in sound trees.

ii. By observations about logs from wind-damaged trees of *Fagus crenata* BLUME at timber-yards and wood-working factories near Hakodate, it is considered that there is no important trouble over estimate from outward inspection.

149. Studies on water-borne wood preservatives. Part 4. On the application of the cup method for assaying the inhibitory effect of water-borne wood presetvatives on the growth of fungi. Takeo SHIBAMOTO, Yoshiyuki INOUE and Yoshihisa KENJO (Journ. Jap. Wood Research Soc., 4, 165–170, 1958)

For the evaluation of wood preservatives, the agar method using culture tubes or Petri dishes and the wood block method of sawdust or soil or quartz sand culture have been used. In a recent publication, a simple assay method of penicillin was given. This cup method has already been used in the investigation of the other antibiotics, and it may have far and wide application in this field. We applied this cup method for testing the effect of the water-borne wood preservatives under some modifications as follows:

An agar medium was inoculated by pouring a pellet suspension produced by shaking culture of the wood-rotting fungi (*Poria vaporaria, Trametes sanguinea* and *Irpex lacteus*). Short open-ended cylinders of stainless steel were placed on the surface of agar and the test solutions containing fluorine compounds or nitrophenols to be assayed were added to the cylinders. After incubation, the surface of the agar became covered with a confluent fungal growth, except for a circular zone around each cylinder where the solution of fluorine compounds or nitrophenols had diffused out. In this way the inhibiting zone, which prevented the growth of the mycelium without killing it, was determined. Moreover, the effect of fluorine compounds and nitrophenols were tested by the wood block method described in JIS A 9302.

Some data obtained were as follows:

1) The pellets of mycelium of *Poria vaporaria, Trametes sanguinea* and *Irpex lacteus* were obtained adequately by shaking culture.

2) Application of the cup method for testing the inhibitory effect of fluorine compounds and nitrophenols on the growth of the wood-rotting fungi was investigated. The effects of magnesium fluoride, cryolite and fluoroacetoamide were not observed. The relative inhibitory effects of some nitrophenols were as follows: sodium 4,6-dinitro-*o*-cresylate>sodium 2,4-dinitrophenate>*p*-nitrophenol. But, the effects of sodium 2,6-dinitro-*m*-cresylate, sodium *o*-nitrophenate and *m*-nitrophenol were not observed.

3) In the case of nitrophenols, the clear inhibiting zones were obtained, but in the case of fluorine compounds were not obtained.

4) Loss in weight of wood blocks treated with fluorine compounds and nitrophenols, by the method described in JIS A 9302, was seen.

Part 5. The effects of pH of treating solution of chloro- and nitrophenol preservatives upon antifungal activities. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Jap. Wood Research Soc., 4, 171–175, 1958)

The water-borne wood preservatives, the reactivity for wood and wood-rotting fungi, the adsorption at total surface of wood, and the permeability of wood cell membrane are of great importance as well as the fungitoxicity, and all these properties will

be considered mainly resulting from the ionization of the treating solution.

The polor groups in the semipermeable membrane possess electrical charge so that charged ions should permeate poorly because they might be grabbed up. Ionization is also related to fat/water partition, free acids are more fat soluble than sodium salts, and sodium salts of compounds are somewhat less toxic than the free acids, because they are better ionized.

The differential effects of ionized- and non-ionized molecules of chloro- and nitrophenols on the wood-rotting fungi and molds were studied. The results obtained were as follows:

1) By the cup method using pellets of wood-rotting fungi and by the poisoned agar method (dilution method), the great effects of pH upon fungistatic activities of 2,4-dinitrophenol, 2,4-dichlorophenol and 4,6-dinitro-*o*-cresol were observed. In most cases, chloro- and nitrophenols were more toxic at low pH, and as the acidity of the media increases, ionizatoin increases, but fungistatic effectiveness of compounds diminishes.

2) Using mixed suspensions of *Penicillium citrinum* and *Aspergillus flavus*, the degree of antifungal activity of *p*-nitrophenol for preservation of bamboo stem were examined. Treating solution at low pH was more fungicidal than at high pH of the same *p*-nitrophenol concentrations.

Part 6. Prevention of mold growth on bamboo stem treated with antifungal chemicals. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Jap. Wood Research Soc., 4, 237-242, 1958)

In a previous paper, the prevention of mold growth on bamboo stem treated with various chemicals was described. In the present report, further observations of the fungal resistance of bamboo stem treated with some phenolic antifungal chemicals were examined in laboratory and field. The bamboo used was Mousou-Chiku (*Phyllostachys pubescence* MAZEL). In the laboratory test, the method of JIS Z 2911 was applied for testing fungal resistance. As an inoculum, mixed spore suspension of *Aspergillus flavus* ATCC 9643 and *Penicillum citrinum* ATCC 9848 were used. Each fungus was cultivated on potato agar medium at 28°C for 10∼15 days, and the small portion of their spores formed was taken up and suspended in sterilized distilled water. The two kinds of fungal spore suspensions were then mixed and poured on the surface of the treated test pieces ($5\times2\times0.5$ cm) which were placed on the potato agar medium in the petri-dish and cultured for 14 days. The test pieces were previously boiled for 30 minutes in the solution of testing chemicals. In the field test, the test pieces (about $20\times6\times1$ cm) were placed in the cellar, since the environment there seemed to be more conducive to mold growth, for 50∼65 days in the warm and rainy season.

The results obtained were as follows:

1) In the laboratory test, when the pH of treating solution is low, the antifungal activity is greater.

2) In the field test, 0.5% *p*-nitrophenol (pH 4.0∼4.4) is more effective as compared with 2% sodium pentachlorophenate (pH 8.4∼8.6) which is commonly used as an antifungal chemical for bamboo stem. No growth of molds were found on the surface of test pieces treated with *p*-nitrophenol and sodium pentachlorophenate, but a mold *Cladosporium herbarum* was detected in the vessels.

Part 7. The result of evaluation of wood preservatives against decay of wood. (Part 1

Takeo SHIBAMOTO, Yoshiyuki INOUE and Yoshihisa KENJO (Journ. Jap. Woold Research Soc., 5, 31-38, 1959)

The standard wood block method recognized as Japanese industrial standard A 9302 was slightly modified and applied for comparing the effectiveness and permanence of 48 kinds of preservatives. The outline of the method is as follows: Test block (20 ± 0.5 mm $\times 20\pm0.5$ mm in cross section and 10 ± 0.5 mm in longitudinal section) is sapwood of *Cryptomeria japonica* D. DON., with annual rings from three to five per 10 mm, no visible evidence of defect, and test fungus is *Poria vaporaria* COOK. The culture mediums are the following: 0.4 g glucose, 0.08 g peptone, ca. 80 cc water and the air-dried 40 g of 10~60 mesh sawdust of beech (*Fagus crenata* BLUME) in the 300 cc wide-mouth Erlenmeyer's flask, sterilized by steam intermittently. Small portion of the mycelium grown on agar slant is inoculated and incubated at 26 ± 2°C, over 70% relative humidity until the medium is covered by mycelium (ordinary 10 days). The test blocks, which were previously dried and weighed to the 0.01 g, are placed in a beaker and held in a vacuum desiccator at the pressure 50 mmHg for 10 minutes. The test solution of preservatives prepared in appropriate concentration is poured into the beaker through the top of desiccator. Immediately, vacuum released at atmospheric pressure and kept for 10 minutes. The test block must be completely filled with the preservative solution. The standard amount of preservative solution absorbed by the block is 250 ± 10% for water-borne preservatives, and 200 ± 10% for oils and oil-borne preservatives. After the impregnation, weathering procedure is carried out (repeated 10, 15 or 25 times): The block is kept at 60 ± 2°C for 24 hours and leached at 25 ± 3°C for 1 hour with running water from 2 to 3 *l* per minute. After the block is dried and weighed, it is placed, with a cross section face down, on the surface of fungal growth in the culture medium mentioned above, and exposed to the attack for 90 ± 2 days. Decaying test being finished, brush off carefully the mycelium on the surface of the test block. Then, the block is dried (60 ± 2°C, 48 hours) and the loss in weight by decaying is measured.

The results obtained are as follows:

1) In the case of Malenit and some allied mixtures, the effect of addition of sodium 4,6-dinitro-*o*-cresylate in place of dinitrophenols and chromium trifluoride was somewhat remarkable.

2) Potassium bichromate and disodium hydrogen arsenate are less toxic than fluoride. In this condition, they are considered to be negative toxic.

3) Of the preservatives containing arsenic compounds, the effective value of sample No. 22 containing no sodium 4,6-dinitro-*o*-cresylate was quite poor. It seems that the leachability of sodium fluoride was not much reduced by the addition of potassium bichromate, but not in the case of disodium hydrogen arsenate; presumably it combined with bichromate to yield chromium arsenate. Even if chromium cryolite and chromium arsenate were formed, they will not be so poisonous at low concentration. At first, the next reaction will occur by the reduced action of wood.

$$K_2Cr_2O_7 + 2Na_2HAsO_4 + 2H_2O \rightarrow 2CrAsO_4 + 4NaOH + 2KOH + 3O$$

4) In the fire-retardant preservatives, the toxic effect of addition of diammonium hydrogen phosphate or ammonium sulphate was quite negative.

5) The effective values of 0.6% Boliden salt S 25 and allied salt mixtures were negative. *p*-Nitrophenol, added in Boliden salt, promoted the preserving effectiveness at low concentration of their salt and inhibited the growth of molds.

6) Sodium *p*-nitrophenate or sodium pentachlorophenate, copper sulphate and potassium bichromate dissolved in ammonia water have great effectiveness after 15~25 times of weathering procedure.

Part 8. Relation between the hygroscopicity and the fixation phenomena of treated wood. Takeo SHIBAMOTO and Yoshiyuki INOUE (Journ. Jap. Wood Research Soc., 5, 86-82, 1959)

Hyogroscopic property of non-volatile, water-borne preservatives is of practical importance and can be ascertained by their critical relative humidity which corresponds to the specific vapor pressure of saturated solution of that substance. As to a mixture of soluble chemicals, Elder laid down the hypothesis: critical relative humidity of a mixture of several soluble solids is equal to the product of the critical relative humidity of several components. In this paper, the critical relative humidity at 37°C was determined by two methods. One is the dry powder method which is suitable for measuring the critical relative humidity of the substance itself, and the other is the saturated solution method which is suitable for finding an accurate critical relative humidity of the substance. As the hygroscopic moisture of treated wood has an influence on the corrosive action of metal and on the electric resistance, the relation between the hygroscopic properties of a test block treated with Wolman type preservatives and the fixation phenomena was also examined. Test block (20×20 mm in cross section and 10 mm in longitudinal section) was taken from sapwood of *Cryptomeria japonica* D. DON., and the amount of preservative solution absorbed by the test block was $250 \pm 10\%$. After the water absorption, the test block was dried at 60°C for 48 hours and the absorbed water in equilibrium with atmospheres of different humidities at 37°C was measured.

The results of experiments obtained and observations made are as follows:

1) The critical relative humidity of single substances are in the following order. Sodium 4, 6-dinitro-*o*-cresylate $> K_2Cr_2O_7 \fallingdotseq CuSO_4 \cdot 5H_2O > NaF > Ha_2HAsO_4 \cdot 7H_2O > ZnSO_4 \cdot 7H_2O >$ Tartaric acid $> Na_2Cr_2O_7 \cdot 2H_2O$

2) With regard to the hygroscopicity of Wolman type preservatives, the applicability of Elders hypothesis is observed.

3) The critical relative humidity of insoluble substance, cupric arsenate or chromium cryolite, are not observed.

4) The fixation phenomena of preservatives in wood may be evaluated by measuring the hygroscopicity of treated wood. From this method, potassium bichromate seems to be fixed more easily with disodium hydrogen arsenate than with sodium fluoride, and the fixation period may be considered to be about two weeks.

5) The result above mentioned shows that the moisture absorption period described in JIS A 9305 is not adequate, but 2~3 days may be suitable for test.

150. Metabolic products from aromatic compounds by the wood-rotting fungus "*Polysticus sanguineus*". Acetoacetic acid and α-ketoglutaric acid from the medium containing benzoic acid. Soshio FUKUZUMI, Kyoji MINAMI and Takeo RHIBAMOTO (Journ. Jap. Wood Research Soc. 5, 96–98, 1859)

Acetoacetic acid and α-ketoglutaric acid were caught as their 2,4-dinitrophenylhydrazones in the medium containing benzoic acid, which was cultured with mycelial pellets of the wood-rotting fungus *Polystictus sanguineus*. 2,4-Dinitrophenylhydrazones of acetoacetic acid and α-ketoglutaric acid were identified and estimated quantitatively

by the absorption curve at 400～700 mμ by means of BECKMAN spectrophotometer.

151. **Fractional dissolution curves of nitrated pulps with ethyl acetate-ethanol mixtures. Part 1. The influence of fiber structure on the curve.** Osakazu NAKAO, Hideaki OKUMURA and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan 15, 175–181, 1959)

Rånby et al. had extracted nitrated pulp fibers with ethyl acetate-ethanol mixtures of increased ethyl acetate concentration, and found that the solubility curve thus obtained shows a level (Fig. 1). This fact means the carbohydrates are deposited in the plant fibers as two fractions differ markedly in solubility after nitration. In their investigation, two fractions were denitrated, hydrolyzed to monosaccharides and then analyzed by paper chromatography. This solvent-nonsolvent system gives a swelling of the fibers without destroying the fiber structure, until the concentration of ethyl acetate in mixture becomes about 60% (in volume). Therefore, the shape of this solubility curve can be effected not only by the amounts of easily soluble carbohydrates, but also the fiber structures, mainly outer layer of the cell wall and fine structure.

The purpose of this investigation has been to establish what effect the fiber structures and average degree of polymerization of pulp could have on the shape of the fractional dissolution curve. The following samples were prepared for determining the solubility curve:

1. F-pulp fibers, which had been immersed in water, frozen and thawed, were prepared. This can be seen as destroyed of outer layer of cell wall or loosen fiber framework without the degradation of cellulose chain and the change of crystalline region.

2. Acid hydrolyzed pulp fibers were prepared under the mild conditions. This can be seen as degradated of cellulose chain and as destroyed of outer layer of cell wall without any change in crystalline region.

Two kinds of acid were used for hydrolysis of pulp fibers, namely sulfuric acid and lignosulfonic acid. Acording to the investigation by Samuelson, the latter had "screen effect", then the outer layer of cell wall can be attacked more severely with lignosulfonic acid than with sulfuric acid.

3. Mercerized pulp fibers were prepared. This can be seen as disordered of the crystalline region without degradation of cellulose chain. A part of easily soluble carbohydrates goes into solution during mercerization treatment.

4. Linters pulp (Li.P), dissolving sulfite pulp (DSP), dissolving sulfate pulp (DKP), bleached paper sulfite pulp (BSP) and bleached paper sulfate pulp (BKP) were also used as original pulps.

Mitchell's mixture was used for nitration of pulp fibers and the fractional dissolution was performed in the way Rånby did. The shape of solubility curve has been expressed by l, h, a and s (Fig. 1).

As a result, it was found that l and a are affected by fiber structure, namely outer layer of cell wall and fine structure. When the outer layer of cell wall was destroyed or lateral order of cellulose was lowered, l decreased. In the case of DKP, the recrystallization had occurred during freezing or acid hydrolysis and l increased. h shows the amount of easily soluble carbohydrates in the fiber, and the shape s depends on the correlation between h and character of outer layer of cell wall. And the effect of average degree of polymerization of cellulose chain on the shape of fractional dissolution curve can be seen as subordinated to the fiber structure.

Part 2. The influence of degree of polymerization of cellulose chains on the curve. Osakazu NAKAO, Makoto USUDA and Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan **15**, 271–275, 1959)

In the previous paper, the effects of fiber structure and the average degree of polymerization of cellulose chains on the shape of the fractional dissolution curve were studied. And it was found that the effect of the fiber structure on the shape of the curve is dominant.

The swelling or solubility of the linear polymer is principally the function of both the length of molecules and the state of cohesion of the molecules. In this paper, the effects of the distribution of the cellulose chain length on the shape of the curve have been studied to confirm the results obtained in the previous paper. And the distributions of cellulose chain length of original pulps and the degree of polymerization of each fractional dissolved were determined.

The results obtained showed the fractional dissolution curve does not represent the distribution of cellulose chain length of the pulp, and supported the results obtained in the previous paper. Furthermore, the results obtained in this paper and previous paper are discussed based on the fiber structure model.

152. **Fine structure of native cellulose fibers. Part 1. Change in fine structure of wood pulp fibers with caustic treatment.** Hiroshi NISHIMURA, Osakazu NAKANO, Nobuhiko MIGITA (Journ. Soc. Textile and Cellulose Ind., Japan **14**, 684–688, 1958)

In order to investigate changes in fine structure of cellulose fibers in caustic treatment, a series of samples were prepared from a commercial dissolving sulfite pulp by steeping in 4∼18% sodium hydroxide solutions at 20°C, washing and drying. These samples were subjected to acid hydrolysis, and their crystallinity, leveling off degree of polymerization and moisture regain of the hydrocellulose were determined, as values related with weight, length and width of micells (highly ordered regions) respectively.

The length and width of micells decreased with increase in concentration of steeping solution from 0% to 10∼12% (Fig. 3, 5). This fact shows that transitional area exists in these both directions of micells. The crystallinity also decreased in that concentration range, but it increased again at higher concentration, while the size of micells remained constant (Fig. 2). Therefore it seems that the re-increase of crystallinity is not due to the increase of size of micells, but to the increase in number of micells, though there remains a problem in the methods used here to estimate size of micells.

153. **Effect of alkaline cooking on lignin. Part 1. Occurrence of secondary condensation reaction in lignin.** Atsushi ISHIZU, Junzo NAKANO, Hiromitsu OYA and Nobuhiko MIGITA (Journ. Jap. Wood Researbh Soc. **4**, 176–182, 1958)

We assumed that the increase of the carbon-carbon bond at the C_5 position of aromatic nucleus in lignin molecule during the alkaline cooking is attributed to the condensation reaction between the benzyl cation derived from the benzyl group and the C_5 position excited by the phenolic hydroxyl group. In order to confirm the above assumption various model compounds corresponding to X or Z group was heated with N-NaOH at 130∼160°C. The resulting products were methylated with dimethylsulfate and oxidized with potassium permanganate. If the secondary formation of the carbon-

carbon bond at the C_5 position occurs during the alkaline cooking, isohemipinic acid must be found in the oxidized products. This acid was detected chromatographically only in the case of X group model compounds. It was thus concluded that at least a part of the condensation reaction at the C_5 position of aromatic nucleus during the cooking occurs between the benzyl hydroxyl group of X group and the C_5 position of aromatic nucleus having the phenolic hydroxyl group. Transformation of the lignin B and Z groups to X group was also studied by using model compounds.

154. **Hydrogenolysis of benzyl groups.** Junzo NAKANO, Atsushi ISHIZU, Nobuhiko MIGITA (Kogyo Kagaku Zasshi 61, 1080–1082, 1958)

The method of hydrogenolysis was used to examine the nature of bonds at α-position in the side chain of lignin molecule. In the first place a series of model compounds were subjected to hydrogenolysis. The result shows that the ether- and ester-bonds at α-position are cleaved, but those at β-position are stable. Furthermore, the phenylcoumaran rings are not cleaved under the condition employed by us. Then this method was applied to beech methanol lignin (OCH_3 content: 25.7%) under pressure. By this treatment the methoxyl content decreases to 24.5%. From the result it may be concluded that a part of methoxyl groups in methanol lignin surely exists in the form of benzyl ether.

155. **On the Mechanism of Color Reaction of Ligin. Part 8. MÄULE Color Reaction. (5).** Junzo NAKANO, Kimio HIRAKAWA, Atsushi ISHIZU, Akio KOBAYASHI and Nobuhiko MIGITA (Journ. Jap. Wood Research Soc. 5, 50–54, 1959)

Some experiments were carried out to obtain further information on MÄULE color reaction which is characteristic for discrimination between hardwoods and softwoods. The results may be summarized as follows:

(1) The synthetic lignins were prepared biochemically after the Siegel method and the Freudenberg method. All the synthetic lignins prpared are negative in MÄULE color reaction, although some synthetic lignins prepared from the compounds containing syringyl nucleus are positive in CROSS-BEVAN reaction (Cl_2-Na_2SO_3). These results show that there is a distinct difference between the mechanisms of MÄULE and CROSS-BEVAN color reaction.

(2) $KMnO_4$-HCl treatments in MÄULE reaction unfailingly separate into two steps; first step by the treatment with $KMnO_4$ and second step by the treatment with MnO_2-HCl.

(3) In MÄULE color reaction, H_2O_2-NaOH, NaOCl-NaOH, $K_2S_2O_8$-NaOH and $K_3Fe(CN)_6$-KOH can be used in place of $KMnO_4$ (first step) and $Pb(CH_3CO_2)_4$-CH_3COOH can be used in place of MnO_2-HCl (second step). That is to say, each color reaction given in the combination of those reagents is considered to be a modified MÄULE color reaction.

156. **Researches on the swelling of fibers. Part 5. Relations between the morphological structures and several Properties of the fibers hydrolyzed by acid.** Hayao HOSOI, Tsuneaki KIJIMA, Kenichiro MATSUSHIMA, Yoshiko TANIGUCHI and Nobuhiko MIGITA (Parupu Kami Kogyo Zasshi 13, 320–327, 1958)

In previous paper, relations between the degree of hydrolysis and the morphological structures of fibers were discussed.

In this paper, for the purpose of examining the relations between the morphological structures and several properties of cellulose fibers hydrolyzed by acid, pentosan and the average degree of polymerization of cellulose were determined on the following samples prepared from pine and beech (holocellulose, hydrolyzed holocellulose, holocellulose treated with 5% KOH, holocellulose treated with 5% KOH and then hydrolyzed). The fractional dissolution curves of nitrates of the samples were also obtained by means of ethyl acetate-ethyl alcohol mixtures.

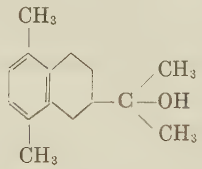
Some differences both in the behaviors of pentosan and in the resistances of the cell walls during the hydrolysis are recognized between pine tracheid and beech wood fiber.

157. Blood-albumin glues. Part 3. Utilization of whale's blood. Hiroshi SAKAGUCHI and Shuhei GOTYO (Journ. Jap. Wood Research Soc. 5, 59–64, 1959)

The adhesive qualities of whale's blood were studied. Eight samples of powdered whale's blood were prepared, and subjected to proximate analyses and to gluing strength determinations. The results obtained showed that whale's blood had distinct adhesive qualities when the blood was powdered in a fresh state with suitable driers. Such powdered blood gave a glue that was sufficiently resistant to hot water. It seems that mixing of a large amount of sea-water into whale's blood deteriorates the potential adhesive quality of the blood. As whale's blood can be collected in the fresh and dense state, and moreover, in large quantities by factory-type whaling ship, the blood of pelagic whales seems to be suitable for preparing a water-resistant glue.

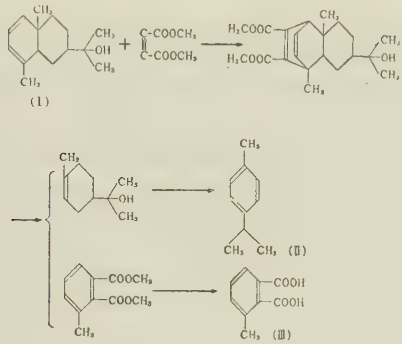
158. Terpenoids. Part 4. The structure of occidol, a new sesquiterpene alcohol from *Thuja occidentalis* L. Yoshiyuki HIROSE and Tomoichiro NAKATSUKA (Bull. Agr. Chem. Soc. Japan 23, 143–144, 1959)

A new sesquiterpene alcohol, $C_{15}H_{22}O$, m.p. 69-70°C, $[\alpha]_D^{25}+163.7°$, named occidol, has been isolated from the essential oil of the wood of *Thuja occidentalis* L. The structure is proposed for it.



Part 6. Further investigation on the constitution of occidentalol. Yoshiyuki HIROSE and Tomoichiro NAKATSUKA (Bull. Agr. Chem. Soc. Japan 23, 140–141, 1959)

Further investigation was carried out to confirm the positions of the conjugated ethylenic linkages and that of the angular methyl group in the proposed formula of occidentalol. Occidentalol was reacted with dimethyl acethylenedicarboxylate at 200°C for 2 hours and the product was then subjected to pyrolysis. From the resulting product, *p-cymene* (II) and 3-methylphthalic acid (III) were identified. These results leave no doubt that occidentalol is correctly represented by (I).



VIII. Zootechnical and Veterinary Science

Abstract Nos. 159—180

159. Histological changes in the mammary gland of guinea-pigs during lactation. Motoo NAITO (Journ. Dairy Res. 25, [3], 392-396, 1958)

Certain histological factors of the mammary gland and the DNA content unit per dry weight were studied at four stages of lactation in guinea-pigs.

The porosity index, the number of alveoli per field, the number of cells per field and the DNA content, showed maximal values at parturition and minimal values at the peak of lactation, whereas the opposite trend was shown by the value for modal alveolar diameter and the number of cells per modal alveolus.

The relationship between these results is discussed.

160. Biochemical studies of the mammary gland function. Part II. Determination of lactose in the mammary gland of rats. Hirokazu SHIMIZU, Yoichi SHODA, Kazuo KURETANI and Saburo UGAMI (Jap. Journ. Zootechn. Sci. 28, [4], 239-241, 1957)

1. An experiment was carried out to determine quantitative changes in the lactose content of the mammary gland of rats in pregnancy, at parturition, during lactation and at weaning.

2. The lactose content was estimated by the semi-microquantitative method of Malpress and Morrison. Litters were allowed to suckle until just before the death of their mothers so that the milk retained in the mammary gland might be removed as completely as possible.

3. Lactose was never found in the mammary gland during pregnancy, but appeared promptly at the time of parturition, namely, initiation of lactation, and the weaning was followed by a rapid decrease of lactose in the mammary gland. These findings were fairly well agreeable with the results reported by KURETANI but were incompatible with the results given by Folley and Greenbaum who demonstrated the presence of lactose in the rat mammary tissue at the beginning of pregnancy and thereafter. This difference seemed to be attributed to the different methods of lactose determination.

4. Since no lactose was found in the mammary gland even at the late stage of

pregnancy, a milk-like substance was shown by histological examination to accumulate in ducts and alveoli at this stage, but it was not true milk. Therefore, the demonstration of lactose in the mammary gland seemed to be a criterion to determine the true lactation in the experiment of induction of lactation.

161. **The effects of forced weaning on the respiratory activity of mouse mammary gland slice.** Hideo MIZUNO and Tateki CHIKANE (Endocrinol. Japon. 5, [4], 265–272, 1958)

The effects of forced weaning on the respiratory activity of mammary slices in mice were investigated. The marked fall in oxygen consumption and R.Q. was observed after weaning. The fact that the non-removal of milk from the gland can be a factor responsible for such reduction of activity and further the possibility that the engorgement of milk might systemically inhibit the mammary activty was shown by the experiment using the technique of unilateral ligature of nipples. However, from the result of the renursing experiment, it was discussed that the lactogenic and mammogenic hormone(s) liberated from the anterior pituitary by the suckling stmulus might be effective for the maintenance of respiratory activity of the mammary gland. In conclusion, the removal of milk seems to play as an important role as the suckling stimulus for maintaining the mammary gland in a functional state.

162. **The effect of locally administered prolaction on the respiratory activity of the mammary gland of rabbit at the corresponding stage to the alveolar formation** Hideo MIZUNO and Motoo NAITO (Endocrinol. Japon. 6, [2], 93–97, 1959)

Japanese white virgin rabbits weighing from 1.8 to 2.3 kg were ovariectomized and the injection of prolactin was begun on the 6th post-operative day. Two pairs of abdominal mammary glands were used in each animal. Prolactin in doses of 1, 3, 5 or 10 iu per day dissolved in 0.3 ml distilled water was injected every other day for a period of 10 days into a galactophore in each of three glands and a remaining gland was served as a control. The animals were killed on the 2nd day after the last injection. The mammary tissues were dissected from injected sectors and cut into fine pieces with scissors. Oxygen consumption and R.Q. were measured by the direct manometric method. There was a fair parallelism between the trends of change of respiratory actvity referring to both wet weight and DNA basis. The oxygen consumption of the gland injected with prolactin was significantly higher than that of the control gland. And it increased nearly linearly with the high dose level of administered prolactin. The carbon dioxide production did not follow the oxygen consumption and resulted in a gradual decrease of R.Q. in the glands injected with prolactin ranging from 1 to 5 iu. However, in the gland injected with 10 iu it increased showing a similar trend to that of oxygen consumption and then resulted in the recovery of R.Q. to the level comparable with the control. The R.Q. was always below unity. In conclusion, it may be possible to say that the changes of respiratory activity obtained in the present study are closely related to the alveolar proliferation, in consideration of the previous reports on histological results (MIZUNO et al. 1955) and on the nucleic acids determination (MIZUNO and NAITO, 1956).

163. **Relation between dairy performance and mammary glands and their stimulators. Part 2. Estrogen level in the blood of goats treated with estrogen for induced lactation: particularly on the method of biological assay.** Hiroshi, NAGASAWA, Yoichi SHODA and Motoo NAITO (Jap. Journ. Animal Reprod. 4, 79–82, 1958)

This study has been done in order to clarify the effect of Euvestin (diethylstilbestrol) sol instead of Euvestin oil for the convenience of practical application, in the case of induction of lactation in goats, which has been studied for the test of the prediction possibility of dairy performance in dairy animals.

The object of this report is to present one modification of biological assay method of small amount of estrogen in the blood, and the changes of estrogen level in the blood of goatlings treated with Euvestin sol or oil, measured by this method.

Animals of group I were injected intramuscularly with 2.5 mg Euvestin sol twice at the interval of 10 days. After 12 days from the 2nd injection, 20 mg Euvestin sol was added two times with 10 days interval. Those of group II were injected subcutaneously with 0.25 mg Euvestin oil daily for 20 days.

(1) Our assay method was almost similar to that of Fujii et al. (1955), which was one of local vaginal response methods. But in considering the persistency of the effect of a test substance and the simplicity of judging so on, a little modification was done; 48, 60 and 72 hours after the 1st insertion of the test substance to the vagina, smears were tested and the points of 3, 2, 1 and 0 were given to the signs of (++), (+), (±) and (−), respectively. Then, the index of the response was determined as the average of the scores of mice for three periods calculated.

According to this method, 2.44 was equal to 1 Vaginal Unit of Allen-Doisy method.

(2) The changes of the level of estrogen in the blood of Euvestin treated goatlings were very fast. The estrogen level of th animals of group I was maximum on the 5th days after the 1st injection and fell down rapidly even after the 2nd injection. Those of group II was maximum on the 14th day after the 1st injection and afterwards fell down in spite of the continuous injection for 20 days.

Further studies may be necessary for clarifying these phenomena mentioned above.

164. Body weight of 12-day-old young as the index of milking performance in mice
Jiro NAGAI and Junzo YAMADA (Jap. Journ. Zootech. Sci. 29, [4], 231-235, 1958)

The growth of young of 12 days after birth and maternal effect of causing variation in body weight of young on the 12th day were investigated in mice at primi- and secundiparity as the first step to study the milking performance of such laboratory animals as mice and rats. Animals used were commercial mice of the Kasukabe strain, which were randomly bred, mating at the ratio of one male to two females. Maternal effects were estimated by means of Bateman's method, using the data in which individual body weight of 12 day old mouse was corrected for the sex and litter size when necessary. Regulation indices and intra-litter correlations were calculated.

Results obtained are as follows;

1) Rearing coefficients were more than 84 and 88 percent in the litter size of 2-9 and 4-11 young born at primi- and secundiparity, respectively.

2) Differences in body weight were found between litter sizes at primi- and secundiparity and between sexes at primiparity.

3) Regulation indices were 0.809 and 1.084 at primi- and secundiparity, respectively.

4) Intra-litter correlation coefficient was 0.94 at primiparity in which body weights were corrected for sex and litter size. The coefficient was 0.96 at secundiparity.

5) Maternal effects of causing variations of both individual and average litter weight were 33 and 35 percent at primiparity, and 65 and 68 percent at secundiparity,

respectively.

165. Variation, hybrid vigor and maternal influence of the mammary growth response of F_1 hybrid mice between inbred strains. Junzo YAMADA (Jap. Journ. Zootech. Sci. 29, [5], 283-281, 1958)

Mice of the bc, C and Swiss inbred strains and F_1 hybrids between bc and C, and bc and Swiss were injected five times with a total dose of 12.5 I. U. of synthetic estrogen every third day beginning at 20 days of age, and their variation of body weight at 35 days and mammary growth response were compared. In these two characters, the potence ratio (F_1—Pm/lP—Pml) of F_1 hybrid was calculated, the presence of maternal influence examined, and its magnitude estimated. The results obtained are as follows;

1) In body weight and mammary growth response, the F_1 hybrids proved to be the most uniform and the inbreds to be the most variable. The randombred mice were between these two groups.

2) The potence ratio of F_1 hybrid mice was more than 1 both in body weight and in mammary growth response. High heterosis seemed to be present in these characters.

3) In body weight, maternal influence was significant at 1% level and the magnitude was 12.6% of total variance. In mammary growth response, maternal influence was significant at 5% level.

According to the mean value of normal skin temperature, the 28 points were classified into the following groups.

166. Studies on the surface temperature of skin in domestic animals. Part 1. Skin temperature of the horse. Toshio OKABE, Toyoichi SUGIYAMA and Sumio OHI (Jap. Journ. Zootech. Sci. 29, [2], 122-128, 1958)

To study temperature distribution in the skin of the horse, skin temperatures were measured at 28 different points in 12 horses kept at the ambient temperature of 20°C. Th results obtained are as follows.

According to the mean value of normal skin temperature, the 28 points were classified into the following groups.

skin temp. (°C)	body	head and neck	foreleg	hind leg
33.0-31.0	tailhead			
31.0-29.0	auscultation point	root of ear		
30.0-28.0	belly, side, back	neck		
29.0-27.0	rump		shoulder-joint, forearm	upper thigh, leg
28.0-26.0		forehead	knee, shank, flexor tendon, fetlock, ankle	hock, shank, flexor tendon, fetlock, ankle
26.0-23.0		muzzle		
24.0-21.0			wall of hoof, bulb of hoof	wall of hoof, bulb of hoof

Furthermore, in order to ascertain the effect of atmospheric temperature on skin temperatures, skin temperatures were measured in 169 horses kept in the range from −5°C to 30°C. The principal results obtained are as follows.

1. The very significant effects of atmospheric temperature on skin temperature were confirmed.

correlation coefficient r	body	head and neck	fore leg	hind leg
0.40–0.65	tailhead	root of ear		
0.65–0.80	auscultation point			
0.75–0.85		neck, forehead		upper thigh
0.80–0.90	belly, rump	muzzle	shoulder-joint	
0.85–0.93	back, side		fore arm, knee, ankle	leg
0.89–0.96			shank, flexor tendon, fetlock	hock, shank, flexor tendon, fetlock, ankle
0.93–0.99			wall of hoof, bull of hoof	wall of hoof, bull of hoof

2. According to the correlation coefficients between skin temperature the measured points were arranged in the following order.

3. The correlation between normal skin temperature (at the ambient temperature of 20°C) and atmospheric temperature-skin temperature correlation coefficient was calculated: $r = -0.78$. Therefore, it seems that the higher the normal skin temperature is, the smaller becomes the effect of atmospheric temperature on skin temperature.

167. Electromyographic studies on the function of the skeletal muscle. VI. Characters of the discharge-interval-time-series of a single NMU. Part 4. The τ-S curve of the dog. Shin-ichi NOMURA (Jap. Journ. Vet. Sci. 20, [5], 223–229, 1958)

It is already a well-known fact that the NMU of the skeletal muscles in man is composed of two groups of quite different properties, that is, the K- and T-NMU. In animals, however, numerous studies have been made without reaching any decisive differentiation of the NMU, because of the difficulties in inducing the animals to a voluntary muscular movement. In maintaining a certain standing posture, which is regarded as one of the fundamental stances in the research on the function of the skeletal muscles in animals, the NMU obtained has always presented the T-NMU. The present study has been attempted to clarify the fact that the muscular movement of animals is maintained not only under the action of the T-NMU, controlled by the subcortical or spinal nervous system, but also under that of the K-NMU, which has been evoked by the activity of the motor area in the cerebral cortex. The procedure is as follows.

1. The sciatic nerves of young dogs and goats were deafferentated by cutting the dorsal root and ganglions on both sides of the spinal segment of L_6, L_7 and S_1. After the serious extension of the hind limbs caused by the operation wore off, the animals were exercised to support the hind half of the body on their feet.

2. Observation of the action potential of the gastrocnemius muscle was repeated every two weeks for period 2 or 3 months. Using the numerous time-series of the discharge intervals of single NMUs, the average τ and the standard deviation S were culculated, and then the τ-S points were graphed for differentiation of the two kinds of NMU. The following results were obtained.

1) The exercise, for two or three weeks after the forced extention of the hind limbs was abolished, enabled the dogs to stand for a few minutes on their tarsal joints with the help of the trainer. The τ-S points then scattered between the K- and T-curve, which is defined at the end of this experiment.

2) After continuation of the exercise for a further period of 2 or 3 weeks, the animals could stand voluntarily on their hind limbs, touching the tarsal joints firmly on the floor. The τ-S points of the time series were clearly divided into two groups, excluding the location of the K-NMU they were markedly biased to the left side.

3) 1 or 2 months later, the dogs learned to stand on their feet, keeping the tarsal joints away from the ground, and to walk and to run around. The $\bar{\tau}$-S points then distributed along the K- and T-curve without overlapping.

4) From the results above-mentioned, it was ascertained that the NMU of the skeletal muscles in the dog is composed of the K- and T-NMU.

5) Nevertheless, it was impossible to discriminate between the two NMU in the case of the goat, in which the legs remained inactive up to the time of their death without any improvement in the mechanical use of the limbs, despite careful management and constant exercise.

Part 5. Relationship between the magnitude of contraction of a muscle and the average discharge-interval of a NMU. Shicichi NOMURA (Jap. Journ. Vet. 20, [5], 231–239, 1958)

From the practical view point of application of electromyograph, it is of great value to determine whether the discharge of NMU increases gradually in frequency to correspond to the progressive increase in the magnitude of contraction in a muscle. Using two adult dogs, the characteristic of each skeletal muscle was studied during quiet standing or while the animals were drafting loads on variously inclined board.

It has been proved that the magnitude of contraction in the gastrocnemius muscle increases gradually, when an adding series of load is chosen, combined with the increase of the inclination of the standing board. Statistical analysis was made on the relationship between the magnitude of the integrated values of the action potentials and the averages of the discharge-intervals of single NMUs, obtained simultaneously from the same muscle. When the average of the discharge-intervals are plotted against the integrated values of the action potentials, the resulting diagram indicates a tendency toward the decrease of the average interval to occur with the larger magnitude of the integrated value.

Nevertheless, a linear relationship is found only in the range of relatively weak contraction. The regression equation departs from the linearity unless the integrated values are transformed to a logarithmic scale. This result means that the regression equation between the integrated values and the average discharge-intervals is a ex-

potential function.

When, however, the magnitude of contraction is determined by a single average of the discharge-intervals of a NMU, it is not possible to give correct information. This is because the individual average intervals are distributed over a wide range, even though the shortening of a muscle is kept at a constant strength. In practice, therefore, it is necessary that the preliminary experiments are carried out to determine the fiducial limits, at a probability of 0.95 or 0.70, of the average intervals corresponding to the progressive increase of the magnitude of contraction, that is, a control chart would be needed.

The ranges in the control chart must be chosen so that there is no overlapping between the adjoining limits ;for example, the range from 60 to 70 ms would correspond to a strong, that from 70 to 80 ms to a moderate contraction, and so forth. An estimate of the strength of a certain muscular activity is made by plotting several numbers of the average intervals of NMU occurring in the same muscle, and obtained during an unchanged contraction, on the control chart. Accordingly when the range involves a large number of the plotted averages, the strength of the contraction of a muscle can be determined.

168. On the delayed implantation in the lactating pregnant rat. Part II. The effect of progesterone. Yoshinaga & Toshiro Hosi (Jap. Journ. Animal Reprod. 4, 107-108, 1958)

Implantation of the fertilized ova fails to take place at the normal time and delays for some days if the rat is lactating.

Since the daily 5 mg of progesterone injection was confirmed to be sufficient to induce ovo-implantation in the castrated rats, we tried to normalize the delayed implantation in the lactating rat with this dosage. The injecions were started on the 1st, 2nd, 5th, 6th or 7th day of pregnancy and continued until 8th day. On the 9th day uterus was examined whether the implantation occurred or not.

When the injections were started before the 6th day, implantation could be induced in almost of all rats. But those which the injections were started on the 7h day, resuled in delayed implantation and their gestation peirods prolonged by 1 or 2 days.

Thus progesterone was effective to normalize the delay in implantation in the lactating rats, even if the injection was started only 1 or 2 days before the normal implantation time.

169. The effect of estrogen on the ovulation time in the cyclic rat. Yutaka Toyoda & Teiichi Eto (Jap. Journ. Aniaml Reprod. 4, 157-163, 1959.)

Using 4-day cyclic female rats of Wistar strain, the shift of ovulation time was investigated following single injections of estrone (1.5-500 μg) at the various stages of the estrous cycle.

Results obtained are as follows:

1) Estrogen delayed the succeeding ovulation by one to three days when it was administered on the day of estrus or on the evening of the day of proestrus, the effectiveness being more remarkable with higher doses.

2) 50-500 μg of estrone, administered between 5 a.m. and noon of the day of early diestrus, advanced the succeeding ovulation by one day in 10 out of 31 animals.

3) Ovulation time was not affected by estrogen treatments on the evening of early diestrus, on the day of late diestrus or on the morning of the day of proestrus.

4) In all cases, even when the ovulations were effectively advanced or delayed, they took place in the midnight. Therefore the ovulation seems to shift from the normal ovulation time by 24 hours as a unit period.

The implications of these results were discussed in respect to the regulation of the gonadotrophic secretion in the estrous cycle of the rat.

170. Some effects of ovarian hormones on the fluctuation of ovulating hormone content of rat hypophyfis. Part II. The change of ovulating hormone content of rat hyphysis stimulated by the hypothalamus. Teiichi ETO, Yutaka TOYODA and Toshiro HOSHI (Jap. Journ. Animal, Reprod. **4,** 83–85, 1958)

The ovulation of rat was inhibited by hypophysectomy performed during 1 p.m. to 3.30 p.m. of the day of proestrus, when the neural mechanism of hypothalamus provokes of the release of ovulating hormone.

After this period, the hypophysectomy decreased the inhibitory effect on ovulation with the lapse of time, and after 5 p.m. almost all the rats ovulated in spite of the hypophysectomy.

At the same time, ovulation inducing capacity of the hypophysis removed at the operation was determined by implantation of them to diestrous rats.

The failure of the inhibitory effect of the hypophysectomy closely correlated with the loss of hte ovulation inducing capacity of the hypophysis.

From this result, it was ascertained that the loss of the capacity can be used as the criterion of the precipitation of ovulation.

Part III. The fluctuation of ovulating hormone content of rat hypohysis in the estrous cycle. Teiichi ETO, Yutaka TOYODA and Toshiro HOSHI (Jap. Journ. Animal Reprod. 4, 86–88, 1958)

The ovulating hormone content of the hypophysis of the rat at various stages in the estrous cycle were estimated by means of the ovulation inducing reaction of diestrous rat by implantation of the pituitary emulsions.

The content began to rise markedly in the afternoon of late diestrus and reached the maximum level where it contained more than twice of the hormone of late diestrus, at 10 p.m. of the day of proestrus.

Thereafter, a sudden and complete loss of the content occurred at 6 p.m. of the day of proestrus. This abrupt fall was apparently the results of the activation of the hypophysis by the neural mechanism of the hypothalmus.

171. Ovulation test in mice as a criterion for the bioassay of HCG Teiichi ETO, Takesh MATSUMOTO and Toshiro HOSHI (Jap. Journ. Animal Reprod. 4, 121–122, 1958)

The induction of ovulation in mature diestrous mice by single subcutaneous injection of HCG was proposed as an useful bioassay of the gonadotropin.

Advantages of this method are as follows:

1. This is highly sensitive and could detect as little as 0.4 MU of HCG, and also its dose-response curve is steep.

2. The time required for the test is quite short; Judgment can be made only 20 hrs. after the HCG injection.

3. The criterion of the positive response, i.e. detection of tubal ova induced by HCG injection, can be made quite easily by every one without any mistake.

4. By using of adults, troublesome precautions which need in immature animals, such as to keep the uniformity of somatic growth, can be eliminated, and a sufficient number of test animals can be easily collected at a time.

172. **Influence of copulatory stimuli on uterine motility to transport the sperm into uterus of the rat. Part I. Effect of copulation on eliciting pseudopregnancy and uterine motility.** Shoso OGAWA (Jap. Journ. Animal Reprod. 4, [3], 127–129, 1958)

Coital behavior in the successive copulations of the rat, coital frequency required for induction of the seminal ejaculation and pseudopregnancy and effect of the copulatory stimuli on the uterine motility were studied with the following results.

1. Unlike the other animals, the rat performed frequent copulations not following ejaculations. The ejaculation took place at least after the successive copulations more than 6～22 times in this experiment.

2. The pseudopregnancy was not induced until the female received the similar copulatory stimuli required for induction of ejaculation; 6～21 times of the copulatory stimuli.

After more than 13 times of successive copulations, the uterus temporarily showed a tendency to contract more intensively than that of non-copulated animals.

Part II. Uterine motility of rat kept in various conditions. Shoso OGAWA (Jap. Journ. Animal Reprod. 4, [3], 130–132, 1958)

Keeping the rats under various laboratory conditions, the uterine motility was investigated by recording the situation on the kymographion.

1. When the animals was anesthetized with the ether inhaltation, the uterus showed rhythmical contractions, which were decreased or almost inhibited by regaining of consciousness and accompanying excitement from the anesthetic condition. This inhibited uterine motility could be recovered and showed the rhythmical contractions, by introducing the animals again into the anesthetic condition.

2. After removing the adrenals, the uterus of the animals regained consciousness from the anesthesia, continued the similar contractions to those of etherized animals.

3. Uterine motility in the adrenalectomized animals was inhibited by dropping of adrenaline solution of the uterine horn.

From these results, it is suggested that the suppression of uterine motility in the animals kept in the exciting condition, might be due to the increased discharge of adrenaline caused by ache and fear.

Part III. Effect of oxytocin on uterine motility of the rat kept in various conditions. Shoso OGAWA (Jap. Journ. Animal Reprod. 4, [3], 133–135, 1958)

Administration of the solutions of oxytocin 1/1500～1/1800 IU/cc on the uterine horn in situ. of etherized animals resulted in the increase of the frequency and the tonus of the uterine contractions. On the other hand, the lower concentrated solution of oxytocin resulted in slight increase of frequency of the contractions.

The rhythmical contractions brought by the administration of 1/800 IU/cc oxytocin solution were inhibited by the simultaneous administration of the solution containing 0.5/cc or more diluted adrenaline on the uterine horn.

The uterus of of unanesthetized animals which suppressed its motility, could induce the rhythmical contractions by the administration of 1/800 IU oxytocin solution.

But, in this case, the contractile behavior was inferior to those of the uterus treated with the same concentration of oxytocin solution in the etherized animals. The results may indicate that, since the uterine contractility is inhibited by the action of a large quantity of adrenaline discharged by ache and fear in such a exciting condition, the stimulating effect of oxytocin on the uterine motility might be decreased.

Part IV. On the sperm migration into uterine horn and the conception rate in the animals kept in various conditions. Shoso OGAWA (Jap. Journ. Animal Reprod. 4, [3], 137–140, 1959)

This study was conducted to investigate the influence of various laboratory conditions on the migratory behavior of the sperm in the genital tract in the rats, and also to investigate the effect of the injection of oxytocin on the conception rate of rats kept in various laboratory conditons after the artificial insemination of semen into vaginal.

1. The injection of oxytocin to the animals under ether anesthesia resulted in rapid migration of sperm into the uterine horn after the deposition of semen into vagina.

In the animals etherized only, sperm migration nto the uterne horn decreased compared wth the former case, but a considerable number of sperm cells was found in the uterine horn at fifteen minutes after the deposition.

The artificial insemination of semen in the adrenalectomized animals kept in concsious condition, showed the similar result to those obtained in the etherized animals.

In the animal of which adrenal was not removed and kept in conscious condition, the deposition of semen resulted in the poorest sperm migration into the uterus compared with those in the etherized, the oxytocin injected and the adrenalectomized animals.

2. A fairly good rate of conception in the rats after the deposition of semen into vagina was obtained using the injection of oxytocin. The etherized animals showed the fairly good conception by injection of much smaller quantity of oxytocin than that required for the none etherized animals.

It is noteworthy that even in the intravaginal insemination, the rat is able to impregnate, if its uterine motility is stimulated by some treatment, such as, by oxytocin injection.

173. **Studies on the motility of accessory sexual organs in the male rat. Part I. Effects of castration and administration of androgen on the motility of accessory sexual organs in situ.** Shoso OGAWA (Jap. Journ. Animal Reprod. 4, [4], 145–148, 1959)

The motility of accessory sexual organs and of muscular group attached to penis in the adult Wistar strain rats under the ether anesthesia were observed in vivo. The results are as follows.

1. In the normal animals, spontaneous intense spasmodic contractions of the accessories occurred, having a serial coordinate transmission mode; Contraction commenced at the epididymal end of vas deferens, overspread to its peripheral end (as if it expelled the semen). It was immediately followed by spasmodic contraction of the muscular group in the portion of gland of the ductus deferens and then by that of ventral prostate (in the meantime the vas deferens exhibited three or four times rhythmical contractions). After the prostatic contractions, the muscular group attached to the penis showed spasmidic contractions accompanying that of scrotum.

The serial contractions of the accessories lasted fifteen to forty-five second, and squeezed out a drop of semen contained many active spermatozoa from the penis. The ejaculation-like movement consisted of complicated contractions of accessories terminated immediately after the seminal emission. This movement appeared about four times intermittently with the rest period of 5 or 7 minutes within restricting only 20~25 minutes of the 45 minutes observation period.

2. In the castrate animals, the accessories showed almost similar contractions to those in the normal animals.

The movement occurred intermittently with regular rest periods throughout the whole observation period.

Consequently, total frequency of the movements in the given observation period were much larger than that in the normal animals.

3. In the castrat-androgenized animals, the ejaculation-like movement and the seminal emission were found in some cases but not in all. The occurrence of the movement, however, was rare (once or twice in the forty-five minute observation.).

From these results, it is suggested that the androgen is making play to inhibit the induction of the ejaculation-like movement.

Part II. Influence of the transection of spinal cord on the motility of the accessory sexual organs. Shoso OGAWA (Jap. Journ. Animal Reprod. 4, [4], 149-150, 1959)

The relation between the ejaculation-like movement of accessory sexual organs and the spinal ejaculatory reflex center was investigated, using the etherized animals of which spinal cord transected at various levels. Results obtained are as follows;

1. In the normal animals, the ejaculation-like movement of accessories and the seminal emissions were found after the transection of the spinal cord at the level of eighth or ninth thoracic segement.

In the animals, of which spinal cords were transected at the levels below than the tenth thoracic, or destroyed throughly between the first lumbar and the sacral level, the accessories did not show any movement.

2. In the castrate animals, the accessories did not show any movement after the destruction of spinal canal throughly the first lumbar to the sacral level.

Judging from these results, the following deduction may be drawn;

a) The ejaculation-like movement of accessories in the normal and the castrate rats might be induced by the spontaneous excitation of spinal ejaculatory reflex center.

b) The ejaculatory reflex center would be possessed of its excitability and carrying its role even after castration.

Part III. Some experiments on the role of accessory sexual organs as the receptor in the ejaculatory reflex. Shoso OGAWA (Jap. Journ. Animal Reprod. 4, [4], 151-154, 1959)

The relation between excitability of the spinal ejaculatory reflex center and androgenic control on ejaculatory mechanism was investigated in comparison with the data obtaind in earlier experiments and also possible physiological significance of androgen on the ejaculatory mechanism was discussed.

The results are summarized as follows;

1. Vas deferens, after transection at its epididymal end, showed the ejaculation-like movement, but, after transection of the peripheral end, did not show any motility.

Regardless of the cutting position in the vas deferens, the ejaculation-like move-

ments of gland of the ductus deferens, prostate, muscular group of penile part and scrotum were still found in almost cases.

2. Removal of seminal vesicle (including coagulating gland) resulted in the reduction of the occurrence of the movement. In the animals of which epididymides were distended artificially by injection of physiological saline solution, there was a tendency to increase the occurrence of the movement.

3. The ejaculation-like movement was decreased or almost inhibited by the injection of procaine solution into the tail of epididymis, the prostate and the seminal vesicle.

4. From the findings obtained in this study and in earlier experiments, it is suggested that the androgen plays an important role to maintain the physiological volume of semen by its delicate diural control on the induction of ejaculation (inhibiting the un-necessary movement of reproductive tracts and, at the same time, stimulating of the secretion in th accessories).

174. Note on some cases of malt rootlet poisoning in dairy cattle II. Norimoto URAKAWA, Toyoaki HAYAMA, Iwao SAKAUCHI and Yoshio OHKUBO (Jap. Journ. Vet. Sci. **20**, 187-191 1958)

Cases of malt rootlet poisoning occurring in dairy cattle which had been brought under observation in the autumn of 1954 were researched. Microorganisms in the poisonous food, and the chemical composition and toxicities of each fraction of the poisonous food were investigated and compared with the normal malt rootlet.

(1) No anærobic microorganism, suspected to be the causes of the poisoning, was discovered in the poisonous malt rootlet.

(2) When compared with the normal sample the poisonous malt rootlets were found to contain more fat and non-protein nitrogen.

(3) The toxicities of the two fractions obtained from the poisonous malt rootlets were found to be greater than those of the normal sample when both fractions were injected to the mouse, intraperitoneally. One of the two fractions was an ether soluble portion and the other was an ethanol soluble portion in 1% HCl soluble part.

In view of the above mentioned results and those mentioned in the previous report, it is presumed that no microorganism found in the poisonous malt rootlet caused the poisoning.

The toxic substance that caused the poisoning might be some chemical substances —mixed or natural poisons—occurring in the poisonous malt rootlet.

175. Studies on radiation injury to domestic animals by radioactive materials. Part I. Absorption, distribution, excretion of P^{32} and its irradiation dose of each organ. Osamu MATSUOKA, Miyoshi IKEDA and Yoshio OHKUBO (Jap. Journ. Vet. Sci. 20, [4] 151-156, 1958)

The present experiments were carried out to investigate absorption, excretion and distribution of P^{32} when injected intraperitoneally into chicks and from the results obtained, the dose rate of various tissues irradiated by β-particles from P^{32} was calculated according to the descriptions by Gerald J. Hine et al. (1956) and W. V. Mayneord (1950).

Part II. Effects of internal irradiation on chicks by P^{32}. Osamu MATSUOKA, Miyoshi IKEDA and Yoshio OHKUBO (Jap. Journ. Vet. Sci. 20 [5], 241-250, 1958)

From observations on the biological effects from internal irradiation by P^{32} the

following results were obtained.

The rate of weight gain was inhibited according as the increase in dosage of P^{32} within the range of 1 $\mu c/g$ (min. dose tested) to 10 $\mu c/g$ (max. dose). In chicks received a single administration of doses over 3 $\mu c/g$, deaths occurred, showing the mortality of 80% with a dose of 5 $\mu c/g$ and 100% with 10 $\mu c/g$ in 11-14 days. It was noted that weight of various organs such as spleen, thymus and testicle decreased, but it increased in such organs as liver, kidney and heart. Food intake was inhibited by doses over 5 $\mu c/g$, but water intake was not affected by any dose tested, and there was no remarkable change to be noted in body temperature, respiration rate and heart rate.

Blood hemoglobin and hematocrit values showed a remarkable decrease and in some cases they decreased slightly. The increased value of albumin in plasma was observed the initial period after administration, but a prolonged increase of globulin was observed over a period of twenty days. Blood sugar after administration of P^{32} gradually increased in the course of time. The glucose tolerance test showed delay of recovery on the course of glucose tolerance curve.

It seemed, in general, that the hematogenic organs were affected remarkably by the internal irradiation (P^{32}), but other organs were noticeably less affected.

176. **Studies on the paper electrophoretic patterns of serum protein in schistosomiasis japonica.** Shin OKOSHI, Isamu TOMODA, Manpei USUI, Mitsuo TAKASHIO and Noritoshi KITANO (Jap. Journ. Vet. Sci. 20, 313, 1958)

Sera from rabbits, dogs and cattle infected with *Schistosoma japonicum* were fractionated in the horizontal bridge filter paper electrophoresis system in order to ascertain the quantitative changes of the serum protein components relating to the degree of infection.

In artificially infected rabbits and dogs, at the early stage of infection, when the schistosomes had not matured, no notable changes in the serum protein fractions were observed. However, following the first appearance of eggs in the feces of hosts, the marked dysproteinemia was observed. In the heavily infected animals, albumin was significantly decreased and the marked increases were in total protein and total globulin, and in consequence the A/G ratio was greatly reduced. Among the globulins alpha 2-, beta- and gamma-globulin were increased, in the dogs; while, in the rabbits, especially gamma-globulin was significantly increased and increases were in alpha 1-, 2- and gamma-globulins, too. In the chronic cases, the serum protein patterns in the dogs showed the significantly decreased albumin and increased beta- and gamma-globulins, especially the gamma, and decreased alpha 1- and 2-globulins; while, in the rabbits, the significantly quantitative increases were observed in total protein, beta- and gamma-globulins, especially the gamma, and albumin and the A/G ratio were markedly decreased as compared with the controls. In the total protein, the tendency of decrease was observed following 60 days after infection in the dogs and 100 days after infection in the rabbits. But, the hypergamma-globulinemia stayed still through the experiment period.

In the cattle, naturally infected, total protein and alpha-globulin showed no significant quantitative differences as compared with the uninfected cattle, though albumin and the A/G ratio were markedly decreased and the significant increases were in beta- and gamma-globulins in the former.

The above serum protein changes, noted in the *Schistosoma japonicum* infected

rabbits, dogs and cattle, could not be regarded as specific. Hypoalbuminemia and hyperglobulinemia are observed in many conditions including the general infectious diseases and liver damages, therefore, it may be thought that the hyperglobulinemia, noted in the schistosomiasis, may be attributed to the result of the damages on liver of hosts, which were induced by deposition of eggs, migration of worms and metabolites of these eggs and worms.

177. Effect of an anthelmintic, Dichlorophen tablet by oral administration on the Canine tapeworm, *Dipylidium caninum*. Shin OKOSHI, Noritoshi KITANO, Isamu TOMODA, Mampei USUI and Mitsuo TAKASHIO. (Jap. Journ. Parasit. 8, 124, 1959)

The teniacidal action of Dichlorophen tablets, which were produced by May and Baker Company in England, was examined by oral administration on the canine tapeworm, *Diphylidium caninum*. The drug has the chemical name of 2:2′-dihydroxyl-dichlorophenyl-5:5′-dichlorophenyl methane, smells of phenol, is not soluble easily in water and of very small crystal. Each tablet contains 0.5 g. of it. The following three examinations were carried out.

1) Toxicity of Dichlorophen tablets on 8 dogs.

2) Teniacidal effectiveness of Dichlorophen tablets to 12 naturally infected dogs.

3) Therapeutic effectiveness of Dichlorophen tablets to 15 naturally infected dogs.

The following results were obtained.

1) Dichlorophen tablets possessed high anthelmintic action against the canine tapeworm and a dosage of about 500 mg. per kg. of body weight of a dog was required in the treatment.

2) Any previous preparation of a dog prior to the treatment or purgatives afterwards were not necessary.

3) In many cases the dogs ceased to pass segments in the feces on the first day or the second following the administration. The teniacidal action of the tablets was a prompt lethal one on the worm, which subsequently underwent rapid degeneration and destruction in the intestine. Therefore the segments were not found out many times in the feces, even when the teniacidal process was carried out successfully.

4) In the experiment on its toxity, only one of eight dogs, which were administrated with a large dose as 2,000 mg./kg., showed temporal diarrhoea. In the treatment test only one case of 15 dogs, which were administrated with doses of 110 to 500 mg./kg. showed slight vomiting. Therefore the tablets are able to be administrated to sick dogs and puppies too.

5) Dichlorophen tablets possessed some anthelmintic action against ascarids of dog, but no action against the other internal helminths, as *Ancylostoma caninum* and *Trichuris vulpis*.

178. Effects of x-ray irradiation on the transmissible venereal tumor of the dog. Masaaki HATAYA, Yasuhiko SHIRASU and Akira TAKEUCHI (Gann 49, 207–318, 1958)

A variety of names has been applied to the transmissible venereal tumor of dogs including Sticker's sarcoma, venereal granuloma, or contageous lymphosarcoma. In therapy, surgical excision or cauterization has been generally employed but incomplete removal frequently results in recurrence. The response of the tumor to x-ray irradiation has been studied.

The quality of radiation employed was as follows: 150 KVp, 3 mA., filter 0.5 mm.

Cu. plus 1.0 mm Al, anode-skin distance 30 cm., field 4×6, 6×8, or 8×10 cm. corresponding to the size of tumor, dose rate 10.56 to 16 r per minute. The dogs were irradiated under general anesthesia with thiopental sodium.

Eight female dogs with spontaneous single or multiple transmissible venereal tumor in the vaginal wall adjacent to the labia, of one to twelve month duration, were used. In each case, a part of the tumor protruded between the labia so that observation and biopsy were convenient. X-ray treatments were administered twice a week over a period of three weeks. Total dosage ranged from 1200 r to 2400 r with six exposures of 200, 300 or 400 r.

Reduction in size and decolorization became evident following the third or fourth treatment. Thereafter, the tumors regressed rapidly. After six exposures (1200 to 2400 r), complete disappearance of the tumors was observed in all cases.

Histologic changes produced by treatment were followed by serial biopsies in all animals. Within three to six hours after the first irradiation, mitoses of the tumor cells almost entirely disappeared in all cases. After this temporary suppression, mitosis reappeared in the tissues biopsied at twelve hours after the first irradiation, but some of these mitotic figures showed abnormality. Since the majority of mitotic aberrations observed twelve to forty-eight hours after the first irradiation occurred during the prophase or metaphase rather than during the anaphase or telophase, it would appear that most of the tumor cells exposed to irradiation died during the stages of prophase or metaphase without completing the subsequent mitotic division. As abnormal mitotic figures, sticky chromosomes, chromosome bridges, asymmetrical division, scattered chromosome fragments or acentric chromosomes were observed.

Cellular and nuclear enlargement of the tumor was seen twelve to forty-eight hours after the first irradiation, and it became more pronounced after the second exposure. The degenerative changes observed in almost all cells following cellular enlargement would suggest that the latter alterations represent an initial manifestation of cellular degeneration induced by irradiation. Deformity of the cell membrane, vacuolization, loosened chromatin network, marginal hyperchromatosis, karyolysis, and pyknosis were observed. Degeneration of tumor cells became remarkably intense after the third or fourth irradiation and many granulocytes, histiocytes, lymphoid cells and plasma cells appeared.

The connective tissue responses observed in the tumor of dogs after irradiation treatment were represented by reactions closely resembling inflammation, with hypertrophy of connective tissue elements resulting in the formation of fibrous tissue. In the present investigation fibroblastic proliferation was observed initially, followed by infiltration of white blood cells during the period when intra-cellular disintegration was most conspicuous; thereafter, degenerated tumor cells disappeared and fibrosis increased.

The present investigation did not demonstrate significant differences in the cellular degeneration or connective tissue proliferation attributable to differences in the dose levels of irradiation. The complete regression of the tumor following exposure to a total dose of 1200 to 2400 r would indicate that the venereal tumor of the dog is highly sensitive to irradiation.

In the present studies an initial temporary neutrophilia was observed in four cases, and an initial slight eosinopenia was observed in five cases. No subsequent leukopenia or eosinophilia could be disclosed. A modest decrease in hemoglobin content

and hematocrit occurred during treatment; thereafter, these values soon returned to or above pre-treatment levels.

In five dogs, permitted to survive, follow-up observations on the results of irradiation have been made for more than two years. No relapse of the tumor has occurred in these dogs. In addition, two dogs have undergone successful pregnancies with normal parturition.

Both clinical and hematological observations indicate that the irradiation therapy in the doses employed was well tolerated.

179. Roentgenography of the canine pulmonary artery with the aid of contrast medium Hikkozo ICHIKI and Kazuo KUROKAWA (Journ. Jap. Vet. Med. Asso. 11, 301-305, 1958)

In preliminary attempts, the exposures for taking the lateral recumbent radiographs of the canine thorax were determined.

Since the ventrodorsal view of the thorax obtained by positioning the dog on its back was not suitable for recording filling of the pulmonary arterial system with radiopaque contrast material, radiographs were taken of the dogs positioned on their left side under general anesthesia.

Organic iodine compounds containing 70% or more of iodine were used as radiopaque contrast medium to show filling of the main pulmonary artery, and left and right pulmonary arteries. Those compounds have given satisfactory results with fewer signs of toxicosis. Radiopaque material was injected into the jugular vein at the rate of 5 to 10 cc per second. The dose of the contrast medium used was as follows:

body weight (kg)	dose (cc)
10	8.0
15	10.0
20	13.0
25	15.0
30	17.5
40	20.0

Since best results were obtained with rapid injection of the contrast medium, the prescribed amount of material was administered into the jugular vein in 1½ to 2½ seconds in smaller dogs, while, in larger dogs, it was injected within 3 seconds.

The insertion of a tracheal tube to which a balloon attached at the outer tip was helpful for taking the radiographs of filling of the pulmonary arterial system with the contrast medium. At the time of exposure, the lungs were inflated by compressing the balloon and a very short period of apnea was obtained. Then, the exposure was made simultaneously with completion of the injection of radiopaque contrast medium.

Excellent radiographs may be obtained, in a small dog with a thoracic width of 10 cm., with exposure time of 0.2 second. In this case, 40 mA is enough for proper exposure with 45 KVp at a distance of 80 cm.

180. Studies on infectious hepatitis of dogs. Part 3. Properties of the virus in dog kidney tissue culture. Takeji IKEGAMI, Shin-ichiro KONISHI and Yuichi OCHI (Jap. Journ. Vet. Sci., 21, 33-38, 1959)

Previously, the authors reported the pathogenicity, immunity and distribution of the infectious canine hepatitis (H.c.c.) virus, in Japan.

The results obtained in the present study with the D43 and D78 strains of the virus of the disease in dog kidney tissue culture were as follows.

1) $TCID_{50}$ of the fluid phase, at various passage levels, were in the range of 10^{-5} to 10^{-7}. Both strains neutralized each other in the cross-neutralization test. The neutralization test, in tissue culture, proved to be the most useful and sensitive method for the diagnosis of H.c.c.

2) The studies on the complement-fixing antigen, the hemagglutinating property and the infective virus in vitro (initial inoculum: 2000 to 6000 $TCID_{50}$) indicated that the infective titer increased on the 2nd day accompanied by the inclusion bodies, reached the maximum titer on the 5th or 6th day after virus inoculation, and after that, remained at the same level with slight fluctuations. On the other hand, the complement-fixing antigen was first detected on the 2nd day after inoculation, when a cytopathogenic effect appeared. And then the complement-fixing antigen titer increased continuously reaching 1:16 on the 6th or 7th day after the virus inoculation.

3) The effects of various temperatures on the virus were studied. It was found that the infectivity of the virus was destroyed by heating at 50°C for 150 minutes or 60°C for 3-5 minutes. At 37°C both viruses were inactivated until the 26th and 29th day after inoculation, respectively. The virus was infective to tissue culture when held at the frozen state with dry ice for 1 year or at 4°C for 9 months. At room temperature the virus kept its infectivity for 10 to 13 weeks.

4) Stability of the virus at 37°C was studied. The results indicated that the complement-fixing antigen, hemagglutinating property and its infectivity were stable, in turn, at this temperature.

5) The virus, at various passage levels, was inoculated into dogs to test their virulence. Between the forty-first and forty-fourth passages, the virus produced an indistinct febrile response and leucopenia after intraperitoneal injection into dogs. Viremia appeared later, but the virus was found to be distributed in many organs, especially to a high degree in the liver, spleen, kidney and lymphatic organs of all dogs infected.